Stimulating Action of Chlordimeform and Desmethylchlordimeform on Motor Discharges of Armyworm, *Leucania separata* WALKER (Lepidoptera: Noctuidae)

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Behavioral and neural excitation of armyworm moths was immediately produced by the injection of each of chlordimeform (CDF; 10 μ g/moth) and desmethylchlordimeform (DMCDF; 10 μ g/moth), *N*-formyl-4-chloro-*o*-toluidine (FCT), and 4-chloro-*o*-toluidine (CT), which are less toxic insect and mammal metabolites of CDF, did not produce any out-standing effect on motor discharges of armyworm moths. Regarding CDF and its metabolites, a correlation existed between their stimulating action on motor discharges and their insecticidal potency. The disturbance of larval feeding activities was clearly demonstrated in silkworm larvae treated with CDF or DMCDF, and it may result from the excessive neural excitation induced by the drugs. MAO-specific inhibitors, iproniazid, niaramid, and tranlycypromine, did not have any out-standing effect on motor discharges and their insecticidal potency were quite low.

INTRODUCTION

The mode of action of chlordimeform (CDF), *N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine, was discussed in several reports and some interesting biochemical findings, *i.e.* inhibition of oxidative phosphorylation and stimulation of ATPase in the cockroach mitochondria,¹⁾ inhibition of the synthesis of DNA, RNA, and proteins,²⁾ and inhibition of monoamine oxidase (MAO) of the mammalian liver,^{3,4)} were made.

Regarding the physiological aspects, CDF caused noncompetitive suppression of acetylcholine (ACh)-induced contraction of rectus abdominis muscle of the frog and additionally caused a slow contraction at higher concentrations.⁵⁾ Furthermore, it was showed that block of neuromuscular transmission in the sciatic nerve-sartorius muscle of frog by CDF is due primarily to the depression of end plate sensitivity to the transmitter.⁶⁾ In cockroaches, the nervous hyperactivity characterized by the

short volley of action potentials was evoked when the CNS was exposed to the saline containing CDF. And in this case, CDF did not block the synaptic transmsion.⁷⁾

In this report, the diverse actions of CDF found in frog nerve-muscle systems were further examined with the intoxicated moth, *Leucania separata*, by the electromyographic observations. Effects of the metabolites of CDF^{8,9)} on motor discharges of armyworm were similarly analyzed from the correlation with their insecticidal potency.

In an attempt to examine the correlation between the MAD inhibition and insecticidal potency, three MAO-specific inhibitors, iproniazid, niaramid, and tranlycypromine, were examined.

MATERIALS AND METHODS

1. Insects

The adult males (1–3 days old) and larvae (3rd and 6th instar) of armyworm, *Leucania*

Table 1 Mortality of armyworm moth injected with CDF, its metabolites and MAO inhibitors.

Compound	Dose ($\mu\text{g}/\text{moth}$)	No. of moth	Mortality (24 hr)
CDF	50	5	100
	10	20	100
	5	15	80
	2.5	20	60
DMCDF ^a	50	5	80
	10	10	50
FCT ^a	40	15	27
	10	20	10
CT ^b	50	15	7
	10	20	15
Niaramid ^a	100	14	14
	10	20	5
Iproniazid	100	14	29
	10	20	0
Tranlycypromine ^a	25	10	40
	10	10	10
50% ethanol-saline		20	10
Saline		20	0

^a: Drug was dissolved in 50% ethanol-saline solution.

25 $\mu\text{g}/\text{moth}$. Other two MAO inhibitors did not show the distinct insecticidal activity.

Symptoms of the intoxicated moths induced by the different dose of CDF can be clearly distinguished. At doses more than 50 $\mu\text{g}/\text{moth}$,

Fig. 2 Excitatory response of armyworm moths to CDF 4 hr after injection.

The moths with flapping wings on both sides are in the phase of excitation. The moth in the center was already in syncope.

both CDF and DMCDF caused syncope of the moth. And at doses less than 25 $\mu\text{g}/\text{moth}$, each of CDF and DMCDF produced the continuous flapping flight (Fig. 2). In the case of 10 μg injection of CDF, this exciting response was continued for 4 to 6 hr. 5 to 8 hr after injection, moths were severely debilitated and reached to the dead condition. Most of moths suffered the injection were died after 10 to 12 hr. FCT temporarily immobilized the moths at the dose of 40 $\mu\text{g}/\text{moth}$, but did not affect

Fig. 3 Motor discharges recorded from the metathoracic leg of the adult male of armyworm injected with CDF and its metabolites.

A, CDF 10 $\mu\text{g}/\text{moth}$; B, DMCDF 10 $\mu\text{g}/\text{moth}$; C, FCT 10 $\mu\text{g}/\text{moth}$; D, CT 10 $\mu\text{g}/\text{moth}$. The numbers indicate the times after injection.

Fig. 4 Motor discharges recorded from the metathoracic leg of the adult male of armyworm injected with MAO-specific inhibitors.

A, niaramid 100 $\mu\text{g}/\text{moth}$; B, tranylcypromine 25 $\mu\text{g}/\text{moth}$; C, iproniazid 100 $\mu\text{g}/\text{moth}$; D, iproniazid 10 $\mu\text{g}/\text{moth}$; E, 50% ethanol-saline solution; F, saline. The numbers indicate the times after injection.

than at the dose of 10 $\mu\text{g}/\text{moth}$. Tremore of the wings was observed in some moths which was injected with CT at the dose of 50 $\mu\text{g}/\text{moth}$, but no effect was produced at the dose of 10 $\mu\text{g}/\text{moth}$. A high dose (100 $\mu\text{g}/\text{moth}$) of iproniazid caused the slight excitation. Although tranylcypromine had weak insecticidal activity, it neither caused the excitation nor other out-standing abnormalities.

The motor discharges recorded from the metathoracic leg of armyworm moths which was injected with each of CDF, metabolites of CDF, and MAO inhibitors are indicated in Figs. 3 and 4. The frequency of motor discharges rapidly increased after injection of CDF and DMCDF (Figs. 3A and 3B) at the dose of 10 $\mu\text{g}/\text{moth}$. Repetitive discharges induced by CDF continued for more than 4 hr. On 5 to 6 hr after injection, the frequency of motor discharges was remarkably decreased accompanying with the prostration of the moths. On the contrary, less toxic compounds such as FCT (Fig. 3C), CT (Fig. 3D), niaramid (Fig. 4A), and tranylcypromine (Fig. 4B) did not produce any appreciable effect on motor discharges. Iproniazid slightly stimulated the motor dis-

charges at the dose of 100 $\mu\text{g}/\text{moth}$, but no effect was produced at the dose of 10 $\mu\text{g}/\text{moth}$ (Fig. 4C, 5D). The effect of the solvent, *i.e.* 50% ethanol-saline solution (Fig. 4E) or saline solution (Fig. 4F), was examined, and no effect was observed.

2. Larvicidal Activity

Injection of CDF at the doses ranging 100–500 μg per larva was followed by typical symptoms of the intoxication within five minutes, but did not produce the mortality of larvae. Symptoms included agony, uncoordination, and hyperactivity. 4 to 5 hr after injection, most larvae were restored from CDF-poisoning. Similar symptoms were induced by the injection of DMCDF at the dose of 100 $\mu\text{g}/\text{larva}$, but no mortality was produced. The larvae injection with 100 $\mu\text{g}/\text{larva}$ of FCT did not suffer from any noticeable effects. Different manners of larval response, which include immediate paralysis and death, were caused by the injection of 100 $\mu\text{g}/\text{larva}$ of CT. Iproniazid neither produced an abnormal response nor a mortality even at the dose of 500 $\mu\text{g}/\text{larva}$.

3. Feeding Repellency

Repellent action of CDF and its metabolites were distinctly demonstrated in the tests against silkworm larvae than in those against armyworm larvae. Both larvae of armyworm and silkworm revealed typical responses, *i.e.* rotation of heads and rapid crawling, on the leaves treated with each of CDF and DMCDF at 2 and 4 hr after the exposures. Twenty-four hours later, the feeding activities of silkworm larvae were strikingly inhibited by CDF and MDCDF (Fig. 5). FCT, CT, and MAO inhibitors did not produce any standing effect on the behaviour of both of armyworm and silkworm and also could not induce the disturbance of their feeding activities. None of chemicals tested have caused the death of armyworm or silkworm at 24 hr after treatment.

DISCUSSION

As indicated in Table 1, CDF shows a high degree of toxicity to armyworm adults. Armyworm and silkworm larvae, particularly at early stages, exhibited a slight disruption of their feeding activities when exposed to CDF. In this case, CDF could not cause the death of both species of larvae. As such disruption was

characterized by exciting responses of the larvae, *i.e.* rotation of heads and rapid crawling, it may be able to speculate that the feeding disturbance in larvae is presumably due to excessive neural excitation. It has been also observed by Kono *et al.* that the accelerated probing frequency in CDF-treated green rice leafhopper¹²⁾ was closely associated with initiation of repetitive motor discharge.¹³⁾ On the contrary, this neural excitation produced by CDF closely related to the insecticidal activity against armyworm adults since the severe prostration of moths resulted from the prolonged exciting behavior. Further application of CDF to the final state of armyworm larvae clearly demonstrated that the larvae have the extreme tolerance to CDF-poisoning.

Yamamoto and Fukami found that CDF caused reversible blockade of neuromuscular transmission and repetitive discharges from single muscle fibres in larval waxmoth.¹⁴⁾ In frog rectus, CDF at the concentration of 10^{-3} M caused a slow contraction and noncompetitively inhibited the ACh-induced contraction at 10^{-4} – 10^{-6} M. And the neuromuscular blockade by CDF was also found in sciatic nerve-sartorius muscle. From these observations, we notice

Fig. 5 A degree of feeding activity of silkworm larvae 24 hr after the exposure to drug-treated mulberry leaf.

All of drugs were treated at the concentration of 1000 ppm.

that CDF has the two different actions, *i.e.* stimulating action on nervous systems and blocking action of neuromuscular transmission in both of insects and frogs. This dual action of CDF possibly depends on the concentration of the drug.

Although iproniazid and niaramid are potent inhibitors of MAO, they have low levels of insecticidal activity, and do not stimulate the motor discharges in hind legs of armyworm moths. Though tranlylcypromine showed a slight insecticidal activity, its action should be distinguished from those of CDF and DMCDF by the difference observed in symptoms and effect on motor discharges. These results do not suggest a correlation between the activity of MAO inhibition and the insecticidal potency of CDF.

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要 約

本報ではクロルジメホルム塩酸塩 (*N*-(4-chloro-*o*-tolyl)-*N'*,*N'*-dimethylformamidine HCl, CDF) およびその代謝物の殺虫性, 惹起する中毒症状および筋電位に及ぼす影響などについて, アワヨトウ (一部カイコを含む) を材料として実験を行なった。また, クロルジメホルムおよび代謝物 デスメチルクロルジメホルム (*N*-(4-chloro-*o*-tolyl)-*N'*-methylformamidine, DMCDF) の示すモノアミンオキシダーゼ (MAO) 阻害作用とそれら殺虫性との相関を明らかにするため, 特異的 MAO 阻害剤についても同様に影響を検討した。その

結果, CDF, DMCDF はアワヨトウ成虫の筋肉に反復興奮を引きおこし, 殺虫性と筋電位刺激作用との間に相関が認められた。また, CDF, DMCDF はアワヨトウ, カイコ両種の幼虫に対しては興奮的な中毒症状を惹起し, 顕著な摂食阻害性が認められた。MAO 阻害剤は殺虫性, 筋電位刺激作用とも著しく弱く, また明確な中毒症状も見いだせなかった。

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