Short Communications

Aryl *N*-propylcarbamates, a Potent Inhibitor of Acetylcholinesterase from the Resistant Green Rice Leafhopper, *Nephotettix cincticeps*

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The green rice leafhopper, *Nephotettix cincticeps* Uhler, becomes a most important pest insect in Japanese rice field. Malathion first used for the control became ineffective since 1962, because of the development of resistance. The resistance was attributed to the increase of carboxyesterase activity as a detoxication mechanism.¹⁾ Then, carbaryl and several phenyl *N*-methylcarbamates have been introduced since 1964, but again the insect is developing resistance to the carbamates since 1969. Important finding was made,^{2,3)} about the mechanism of the resistance: acetylcholinesterase (AChE) obtained from the resistant strain was much less inhibited by several carbamates. AChE activity itself did not change for susceptible and resistant strains, while aliesterase activity was much higher in the resistant strain than in the susceptible strain. The aliesterase from the resistant strain was much more sensitive to the carbamates than that from the susceptible strain. Later studies, however indicated a higher AChE activity from resistant strains than that of susceptible strains.^{4,5)}

Clarification of the cause of decreased inhibition of AChE by carbamate insecticides may be important for providing methods of control of the resistant strain. We have tried various structural modifications of the known carbamates, taking anti-AChE activity as a guiding index, and after all, modification of *N*-methyl group on the carbamyl moiety to *N*-propyl

group was found to be promising. This paper deals with the finding of phenyl *N*-propylcarbamates as a good inhibitor of AChE obtained from the resistant leafhopper, and the nature of interaction of *N*-methyl and *N*-propylcarbamates with AChEs from susceptible and resistant strains (for convenience, the AChEs are referred to as susceptible AChE and resistant AChE, respectively).

It is generally realized that only *N*-methyl or *N,N*-dimethylcarbamates are good inhibitors of AChE and thus, good insecticides. The cause of decrease in inhibitory potency for longer *N*-alkyl compounds was different for the AChEs from different animals as studied by Yu *et al.*⁶⁾ For bovine erythrocyte and honeybee AChEs, it is mainly due to the decrease in carbamylation rate; for housefly AChE, it is mainly due to the decrease in binding energy. For various species, carbamylated AChE recovered much slower when inhibited by longer *N*-alkylcarbamates.

It is, therefore, possible that the decreased sensitivity of resistant AChE to *N*-methylcarbamates may arise from the modification of susceptible AChE. It may be also possible that the increased activity of aliesterase and sensitivity of its enzyme to *N*-methylcarbamates may be involved in the higher I_{50} value for the resistant AChE. However, the latter possibility is eliminated by considering the remarkable difference of I_{50} values between susceptible and resistant AChEs, and the fact

that the I_{50} did not change whether or not the enzyme preparation includes aliesterase activity.⁵⁾ Therefore, we were intrigued to reexamine the effect of change of *N*-alkyl group. The AChE activity was measured by the Ellman method.⁷⁾

Figure 1 shows the inhibition of AChEs from susceptible (S), resistant (RN-N) and more resistant (RN-4) strains by MPMC (3,4-dimethylphenyl *N*-methylcarbamate), and its corresponding *N*-ethyl, *N*-propyl and *N*-butyl analogs. For simplicity, *N*-propyl analog of MPMC is referred to as *N*-propyl-MPMC. S strain is the one collected at Warabi, Saitama and maintained at National Institute of Agricultural Sciences; RN-N and RN-4 are the Nakagawara strain collected in 1969 and 1974, respectively. Only *N*-methylcarbamate is a good inhibitor of susceptible AChE, whereas *N*-methylcarbamate becomes a poor inhibitor of resistant AChE. There is a trend that the more the resistant develops, the more effective the higher *N*-alkyl carbamates become. The inhibition of resistant AChE becomes maximum at *N*-propyl. Not shown in Fig. 1, *N*-allylcarbamate gave the same

Fig. 1 Inhibition of AChEs from susceptible (S), resistant (RN-N) and more resistant (RN-4) strains of *N. cincticeps* by MPMC and its corresponding *N*-alkyl-analogs.

degree of inhibition as *N*-propyl one, but *N*-isopropyl or *N*-branched butylcarbamate did not give good inhibition for resistant AChE. Similar results were obtained by MIPC (2-isopropylphenyl *N*-methylcarbamate), BPMC (2-*sec*-butylphenyl *N*-methylcarbamate), MTMC (3-methylphenyl *N*-methylcarbamate), XMC (3,5-dimethylphenyl *N*-methylcarbamate) and carbaryl (1-naphthyl *N*-methylcarbamate).

The relationship between resistance level indicated by LD_{50} value to *N*-methylcarbamates and anti-AChE activity of *N*-methylcarbamates is shown in Fig. 2. There is a good correlation between resistance development and decreased inhibition by *N*-methylcarbamates. Figure 3 shows the relationship for *N*-propylcarbamates. It is remarkable that the more the resistance develops to *N*-methylcarbamates, the more sensitive to *N*-propylcarbamates the resistant AChE becomes. That is, the inhibition by *N*-propylcarbamates is negatively correlated to *N*-methylcarbamate-resistance level.

The nature of inhibition by *N*-methyl and

Fig. 2 Relationship between resistance level indicated by LD_{50} to *N*-methylcarbamates and anti-AChE activity of *N*-methylcarbamates (pI_{50})

1, MIPC; 2, BPMC; 3, MTMC; 4, MPMC; 5, XMC; 6, carbaryl.

▲ Susceptible (S), ■ Resistant (RN-N), ● More resistant (RN-4).

Fig. 3 Relationship between resistant level of *N*-methylcarbamates (LD_{50}) and anti-AChE activity of *N*-propylcarbamates (pI_{50}).

1, MIPC; 2, BPMC; 3, MTMC; 4, MPMC; 5, XMC; 6, carbaryl,

▲ Susceptible (S), ■ Resistant (RN-N)

● More resistant (RN-4).

N-propylcarbamates was investigated by measuring inhibition constants: binding (K_a), carbamylation (k_2), decarbamylation (k_3) and overall bimolecular reaction (k_i) constants. Table 1 gives the results obtained with two pairs of carbamates: carbaryl and *N*-propyl-carbaryl; BPMC and *N*-propyl-BPMC. With carbaryl, there was a great difference between susceptible and resistant AChEs for K_a value, but not for k_2 value. The ratio of K_a values for resistant and susceptible AChEs was 70, which is comparable to the ratio of k_i and I_{50} values for the two AChEs. The similar trend was observed with BPMC. As shown in Table 2, k_3 is almost the same for resistant and susceptible AChEs, and hence not involved in resistance mechanism. It is obvious that the cause of decreased inhibition of resistant AChE by carbaryl or BPMC, indicated by higher I_{50} value, is due to the decrease of binding energy or affinity of the inhibitor to resistant AChE.

N-Propyl-carbaryl showed almost the same k_2 as carbaryl for susceptible AChE, but the affinity of the carbamate to the AChE indicated

Table 1 Binding, carbamylation, bimolecular reaction constants and I_{50} for the inhibition of AChE from *N. cincticeps*.

Compound	Susceptible (S)	Resistant (RN-4)
Carbaryl		
K_a (M)	8.7×10^{-7}	6.1×10^{-5}
k_2 (min^{-1})	0.7	0.9
k_i ($\text{M}^{-1}\text{min}^{-1}$)	8.5×10^5	1.4×10^4
I_{50} (M)	2.3×10^{-7}	1.7×10^{-5}
<i>N</i> -Propyl-carbaryl		
K_a	1.8×10^{-5}	1.6×10^{-5}
k_2	0.6	2.4
k_i	3.5×10^4	1.5×10^5
I_{50}	1.2×10^{-5}	1.3×10^{-6}
BPMC		
K_a	1.5×10^{-6}	5.0×10^{-4}
k_2	1.3	0.9
k_i	9.0×10^5	1.7×10^3
I_{50}	1.1×10^{-4}	2.9×10^{-4}
<i>N</i> -Propyl-BPMC		
K_a	1.2×10^{-4}	9.4×10^{-5}
k_2	0.6	2.3
k_i	5.1×10^3	2.5×10^4
I_{50}	1.2×10^{-4}	2.5×10^{-5}

The supernatant of 100,000 g, 60 min of the insect homogenate was used. The method of Hart and O'Brien⁹⁾ was used for measuring inhibition constants.

Table 2 Decarbamylation rate constant for the inhibition of AChE from *N. cincticeps* with *N*-alkylcarbamates.

	Methyl	Ethyl	Propyl	Butyl
Susceptible (S)	0.013	0.007	0.002	0.009
Resistant (RN-4)	0.016	0.006	0.014	0.012

The supernatant of 100,000 g, 60 min of the insect homogenate was used. The method of Nishioka *et al*⁸⁾ was used.

by $1/K_a$, was quite low. This is the cause of lower anti-AChE activity of *N*-propyl-carbaryl. For resistant AChE, the affinity of *N*-propyl-carbaryl remained same as susceptible AChE, but the carbamylation rate, k_2 increased almost four fold. This explains why *N*-propyl-carbaryl is better inhibitor for resistant AChE than susceptible AChE. For resistant AChE, *N*-propyl-carbaryl was about four fold than carbaryl in affinity and about three fold in carbamylation rate. Decarbamylation rate was almost the same for both *N*-propyl-

carbamylated and *N*-methylcarbamylated resistant-AChE. Therefore, the combined increase of affinity and carbamylation rate seems to be the major cause of good inhibition of resistant AChE by *N*-propyl-carbaryl.

N-Propyl-BPMC is a poor inhibitor for susceptible AChE due to the lower affinity and lower carbamylation rate than BPMC. In general, the same trend can be seen for carbaryl and BPMC series.

Thus, the available evidences indicate the view that the mechanism of *N*-methylcarbamate resistance is a decreased binding of the carbamates to the active site of AChE, and the modification of the carbamyl group to *N*-propyl increases the affinity and carbamylation rate, thus resulting in the improved AChE inhibition. Search for the case where *N*-propylcarbamates are better AChE inhibitor than the corresponding *N*-methylcarbamates may be interesting from the standpoint of widening our scope for better insecticides.

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要 約

Aryl *N*-methylcarbamates 抵抗性のツマグロヨコバイに有効な化合物を見いだす目的で、アセチルコリンエステラーゼ (AChE) の阻害力を指標にして、種々の構造改変を行なった。その結果、carbamyl moiety の *N*-メチル基を、他の低級アルキル基に変換すると、抵抗性ツマグロヨコバイの AChE を阻害する能力が強く

なることを見いだした。その中でも *N*-プロピル同族体が最も活性の強いものであった。そして *N*-methylcarbamates に対して抵抗性が強くなるとその AChE は *N*-propylcarbamates に対しては逆に感受性になるという負相関の現象を見いだした。つぎに、感受性および抵抗性両系統のツマグロヨコバイの AChE に対する阻害定数の測定を行なった。その結果 *N*-methylcarbamates が抵抗性の AChE に対して阻害力の低下する主要因は K_a の増大、すなわち *N*-methylcarbamates に対する親和力の低下であり、また *N*-propylcarbamates の強い阻害力は K_a の減少 (親和力の増加), k_2 の増加 (carbamylation の増大) という二つの要因によることが明らかとなった。

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