Original Article

N,N-Dialkyl Substituents of 3-Pyridylmethylamines Discriminate between α -Bungarotoxin and Noncompetitive Blocker Sites of Nicotinic Acetylcholine Receptors of *Torpedo* and Insect

Motohiro TOMIZAWA,* Hanako MATSUO, Toru MIYAMOTO and Izuru YAMAMOTO**

Department of Agricultural Chemistry, Tokyo University of Agriculture, Setagaya-ku, Tokyo 156, Japan

(Received April 1, 1996; Accepted August 3, 1996)

The effects of *N,N*-dialkyl substituents of a 3-pyridylmethylaniline on the binding site discrimination between the acetylcholine (ACh) and noncompetitive blocker (NCB) sites of the nicotinic ACh receptors (nAChR) from the *Torpedo* and insect are studied using [^3H] α -bungarotoxin (α -BGT) and [^3H] phencyclidine (PCP) as probes of the ACh and NCB sites, respectively. Increasing the chain length of *N,N*-dialkyl substituents of 3-pyridylmethylaniline from dimethyl to di-*n*-butyl reduces the agonistic potency which contributes to the inhibition of [^3H] α -BGT binding and the stimulation of [^3H] PCP binding. However, this structural modification results in the enhanced affinity to the NCB site attributing to the inhibition of [^3H] PCP binding when the ion channel of *Torpedo* nAChR is opened. A similar relationship is seen in the symmetrically substituted tetraalkylammonium compounds. Like *Torpedo*, the longer alkyl chain of 3-pyridylmethylanilines is preferred as inhibitor of [^3H] PCP binding over as inhibitor of [^3H] α -BGT binding in the insect nAChR. These results suggest that such homologous changes in chemical structure convert the binding site of ligand from the ACh to NCB sites of nAChRs in both *Torpedo* and insect.

INTRODUCTION

Pharmacological effects of ligands involving the function of the nicotinic acetylcholine receptor (nAChR)/ion channel complex have been investigated in the *Torpedo* electric organ.¹⁻³⁾ α -Bungarotoxin (α -BGT)⁴⁾ and phencyclidine (PCP)¹⁾ are known as suitable probes for acetylcholine (ACh) and noncompetitive blocker (NCB) sites of the *Torpedo* nAChR, respectively. In insect, α -BGT also serves as a ligand for the ACh site of nAChR.^{5,6)} The presence of the NCB site in the ion channel of insect nAChR is suggested by the voltage-dependent blockade of the ACh-induced current by PCP.⁷⁾ However, the binding property of PCP to the insect nAChR is different from that to the *Torpedo* nAChR.^{3,7,8)} To approach the NCB site of insect

nAChR, pharmacological profile of the nAChR from insect head is compared with that from *Torpedo* electric organ. Our previous study indicates that PCP binds both to the NCB and ACh sites of the insect nAChR.⁸⁾

Nicotine in an infusion of tobacco leaves is known as an insecticide which interacts with the insect nAChR. Studies on structure-activity relationships (SAR) of insecticidal nicotine and its related compounds have demonstrated that 3-pyridylmethylaniline moiety with highly basic amino nitrogen atom is the essential structural moiety of nicotine for the insecticidal activity and interaction with the insect nAChR. The inferior insecticidal activity of nicotine to certain insect pests is attributed to its cationic nature at physiological pH to interact with the ACh site of nAChR and regulate its penetration through biological barriers.⁹⁻¹¹⁾ The present study has found that the aforementioned 3-pyridylmethylaniline is not merely the inhibitor of [^3H] α -BGT binding but also the inhibitor of [^3H] PCP binding of nAChRs in the *Torpedo* and insect. Therefore, we focus on the SARs of 3-pyridylmethylanilines and the related compounds in terms of their affinities to the α -BGT (designated as ACh site) and NCB sites of the

* Present address: Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720-3112, U.S.A.

** Reprint requests should be addressed.

The first two authors (M. T. and H. M.) contributed equally to this study.

Torpedo and insect nAChR/ion channel complexes.

MATERIALS AND METHODS

1. Materials

[Piperidyl-3, 4-³H(*N*)]phenylcyclidine ([³H]PCP; 49.9 Ci/mmol) and *N*-[propionyl-³H]propionylated α -bungarotoxin ([³H] α -BGT; 68.0 Ci/mmol) were purchased from New England Nuclear Research Products and Amersham Life Science, respectively. Tetramethylammonium iodide, tetraethylammonium chloride, tetrapropylammonium chloride and tetrabutylammonium bromide were purchased from Sigma Chemical Co. Other compounds used in this study were available

from our previous studies.^{3,8,10,11} Chemical structures of compounds employed in this study are shown in Fig. 1.

2. Binding Assays

[³H] α -BGT and [³H]PCP binding to the membranes prepared from the *Torpedo nobiliana* electric organ and honeybee head were described in our previous studies.^{3,8} Dissociation constant (K_D) and maximal binding capacity (B_{max}) of [³H]PCP binding were determined by titration of unlabeled PCP on the binding of 2 (for *Torpedo*) or 5 nM (for honeybee) of [³H]PCP.

3. Data Analysis

Data were analyzed by iterative nonlinear least-squares regression using SigmaPlot (Jandel Scientific Software, San Rafael, CA, U.S.A.) and LIGAND (Biosoft, Cambridge, U.K.) programs. Statistically significant differences between the two groups were determined by Student's *t*-test, and a probability of $p < 0.01$ was accepted as significant.

RESULTS

1. SAR of Pyridylmethamines for the Binding of [³H]PCP and [³H] α -BGT to the *Torpedo* nAChR/Ion Channel Complex

Among the three pyridyl isomers of pyridylmethyl-*N*, *N*-dimethylamine, 3-pyridyl isomer (**2**, Fig. 1) was the

Fig. 1 Chemical structures of tested compounds.

Table 1 Structure-activity relationship of tested compounds for the [³H]PCP and [³H] α -BGT bindings to the membranes from the *Torpedo* electric organ.

Compound no.	[³ H]PCP binding % of control or IC ₅₀ , μ M		[³ H] α -BGT binding IC ₅₀ , μ M
	Alone	With 100 μ M carbachol	
1	(531%) ^{a)}	(94.9%) ^{a)}	> 1000 (11.5%) ^{d)}
2	(1700%) ^{a)}	(98.1%) ^{a)}	340 $\pm$ 48.8
3	(1170%) ^{a)}	(101%) ^{a)}	980 $\pm$ 41.2
4	(271%) ^{a)}	1250 $\pm$ 88.9	> 1000 (20.6%) ^{d)}
5	(96.6%) ^{a)}	134 $\pm$ 7.53	> 1000 (9.29%) ^{d)}
6	(97.1%) ^{a)}	60.8 $\pm$ 7.70	> 1000 (5.89%) ^{d)}
7	(1640%) ^{a)}	1124 $\pm$ 140	410 $\pm$ 27.7
8	(2240%) ^{a)}	(99.8%) ^{a)}	197 $\pm$ 31.5
9	323 $\pm$ 56.3	157 $\pm$ 10.4	> 1000 (16.3%) ^{d)}
10	152 $\pm$ 15.7	3.40 $\pm$ 0.25	> 1000 (14.8%) ^{d)}
11	10.0 $\pm$ 4.89	0.78 $\pm$ 0.14	> 1000 (13.9%) ^{d)}
12	(284%) ^{b)}	204 $\pm$ 28.6	> 300 (13.7%) ^{d)}
13	(304, 73.0%) ^{c)}	33.4 $\pm$ 2.55	> 1000 (9.20%) ^{d)}
14	3.13 $\pm$ 0.50	0.22 $\pm$ 0.01	> 1000 (3.20%) ^{d)}

a, b, c) [³H]PCP bound, percent of control at 300^{b)} and 1000 μ M^{a)} or both.^{c)}

d) Percent inhibition at 300 or 1000 μ M.

IC₅₀ values are means $\pm$ S.D. of three experiments. Numerical values in the parenthesis are means of three experiments with S.D. less than 10%. Dose-response curves of tested compounds on the [³H]PCP binding are illustrated in Figs. 2, 4 and 5, except compounds **1** and **3**. The curves of compounds **1** and **3** are shown in our previous study (see Ref. 12)). [³H]PCP binding was initiated by addition of *Torpedo* membranes, and was terminated after 60 sec incubation. Chemical structures are listed in Fig. 1.

most potent isomer enhancing [^3H]PCP binding compared with those of 2- and 4-isomers (**1**, **3**).¹²⁾ These three isomers did not further increase [^3H]PCP binding as compared with the control in the presence of 100 μM carbachol which has capability to open the ion channel of nAChR. Their actions to potentiate [^3H]PCP binding were attributable to their affinity to the ACh site as indicated by the inhibition of [^3H] α -BGT binding (Table 1). Increasing the chain length of the dialkyl substituents on the amino nitrogen atom of 3-pyridylmethyamines from dimethyl to di-*n*-butyl (**2**, **4**–**6**) reduced the affinity to the ACh site (Table 1). This trend was also observed in their activation effects on [^3H]PCP binding. Compound with dimethyl substituents (**2**) markedly increased [^3H]PCP binding. In contrast, compounds with diethyl, di-*n*-propyl and di-*n*-butyl substituents (**4**–**6**) did not or only slightly increased [^3H]PCP binding (Fig. 2, left). Compounds **4**, **5** and **6** inhibited [^3H]PCP binding in the presence of carbachol, whereas compound **2** did not (Table 1 and Fig. 2, right).

Fig. 2 Effect of increasing the chain length of *N, N*-dialkyl substituted 3-pyridylmethyamines from dimethyl to di-*n*-butyl on the [^3H]PCP binding in the absence (left) and presence (right) of 100 μM carbachol in the *Torpedo*.

The binding reaction was performed under the condition of 60 sec incubation. Symbols represent mean of three experiments with S.D. less than 10%. Numbers on graphs refer to compounds listed in Fig. 1.

Fig. 4 Effect of increasing the chain length of a homologous series of symmetrically substituted tetraalkylammonium compounds from tetramethyl to tetra-*n*-butyl on the [^3H]PCP binding in the absence (left and center) and presence (right) of 100 μM carbachol in the *Torpedo*.

The binding reaction was performed under the condition of 60 sec incubation. Symbols represent mean of three experiments with S.D. less than 10%. Numbers on graphs refer to compounds listed in Fig. 1.

2. Effect of 3-Pyridylmethyl-*N, N*-di-*n*-butylamine on K_D and $B_{\max}$ Values of Carbachol-Stimulated [^3H]PCP Binding in the *Torpedo*

Scatchard plots on 2 nM [^3H]PCP binding stimulated by carbachol in the absence and presence of 75 μM 3-pyridylmethyl-*N, N*-di-*n*-butylamine (**6**) showed the convergence of two lines on the X axis. K_D value in the presence of compound **6** was increased, while $B_{\max}$ value remained unchanged (Fig. 3). Hill coefficient indicated the presence of single binding site for [^3H]PCP at both conditions.

3. Effect of Increasing the Chain Length of Tetraalkylammonium Compounds on the Binding of [^3H]PCP and [^3H] α -BGT in the *Torpedo*

The activation potency of tetramethylammonium compound (**8**) on [^3H]PCP binding was attributed to its affinity to the ACh site as indicated by inhibition of

Fig. 3 Scatchard plots for 100 μM carbachol-stimulated [^3H]PCP binding to the membranes from the *Torpedo* electric organ.

The binding reaction was performed in the absence ($\circ$) and presence ($\bullet$) of 75 μM 3-pyridylmethyl-*N, N*-di-*n*-butylamine (**6**) with 60 sec incubation. Binding parameters are as follows: K_D 123 $\pm$ 2.47 nM, $B_{\max}$ 52.4 $\pm$ 0.49 pmol/mg protein and Hill coefficient (n_H) 1.03 $\pm$ 0.05 in the absence of compound **6**, while K_D 447 $\pm$ 14.3 nM, $B_{\max}$ 58.0 $\pm$ 1.56 pmol/mg protein and Hill coefficient (n_H) 0.99 $\pm$ 0.02 in the presence of compound **6**. Values are means $\pm$ S.D. of three experiments. * Significant difference between control and treatment ($p < 0.01$).

[^3H]- α -BGT binding (Fig. 4, left and Table 1). It did not further increase [^3H]PCP binding when 100 μM carbachol was present (Fig. 4, right and Table 1). When the chain length of a homologous series of symmetrically substituted tetraalkylammonium compounds increased from tetraethyl to tetra-*n*-butyl (9–11), these three compounds inhibited [^3H]PCP binding (Fig. 4, center and Table 1) and became more potent inhibitors of [^3H]PCP binding in the presence of carbachol as indicated by IC_{50} values, although they failed to inhibit [^3H]- α -BGT binding (Fig. 4, right and Table 1).

4. Effect of Replacing the 3-Pyridylmethyl Group with Benzyl Group on the Binding of [^3H]PCP and [^3H]- α -BGT in the Torpedo

3-Pyridylmethyl-*N,N*-dimethylamine (2) markedly increased [^3H]PCP binding, whereas the replacement of 3-pyridylmethyl group with benzyl group (12) only slightly increased [^3H]PCP binding (Fig. 5, left and center). These results were attributable to their affinity to the ACh site as indicated by the inhibition of [^3H]- α -BGT binding (Table 1). In the presence of carbachol, compound 2 did not further increase [^3H]PCP binding as compared with control. In contrast, compound 12 inhibited [^3H]PCP binding in the activated status of the receptor (Fig. 5, right and Table 1). Moreover, *N*-(3-pyridylmethyl)piperidine (7) remarkably increased [^3H]PCP binding. *N*-Benzylpiperidine (13) increased it maximally 304% at 300 μM , then reduced it to 73% at 1 mM (Fig. 5, center). Compounds 7 and 13 inhibited [^3H]PCP binding when carbachol was present. PCP (14) was more potent for the activated receptor as indicated by IC_{50} values in the absence and presence of carbachol. Potencies of compounds having benzyl group as inhibitors of [^3H]PCP binding in the presence of carbachol were higher than those of compounds having 3-pyridylmethyl group (Fig. 5, right and Table 1).

5. SAR of Pyridylmethylamines and Benzylamines for the Binding of [^3H]PCP and [^3H]- α -BGT in the Honeybee

Among three pyridyl isomers of pyridylmethyl-*N,N*-dimethylamine, the 3-pyridyl isomer (2) showed the highest affinity to the ACh site compared with those of 2- and 4-isomers (1, 3). Although all of the three isomers inhibited [^3H]PCP binding, the order of potency of the three isomers on the [^3H]PCP binding did not coincide with that on [^3H]- α -BGT binding (Table 2). Increasing the chain length of the dialkyl substituents on the amino nitrogen atom of 3-pyridylmethylamines from dimethyl to di-*n*-butyl (2, 4–6) enhanced the potency as inhibitors of [^3H]PCP binding, while it reduced the potency as inhibitors of [^3H]- α -BGT binding. Among the 3-pyridylmethylamines, compound 6 demonstrated the greatest inhibition on [^3H]PCP binding and the least inhibition on [^3H]- α -BGT binding (Table 2). The piperidyl analog (7) of 3-pyridylmethylamine showed high potency as inhibitor of [^3H]PCP binding as well as

Table 2 Structure-activity relationship of pyridylmethylamines and benzylamines for the [^3H]PCP and [^3H]- α -BGT bindings to the membranes from the honeybee heads.

Compound no.	IC_{50} , μM	
	[^3H]PCP	[^3H]- α -BGT
1	56.4 $\pm$ 8.33	> 1000 (44.3%) ^{a)}
2	55.3 $\pm$ 5.04	134 $\pm$ 7.70
3	23.2 $\pm$ 0.13	> 1000 (33.7%) ^{a)}
4	4.00 $\pm$ 0.23	195 $\pm$ 4.60
5	1.90 $\pm$ 0.11	264 $\pm$ 26.0
6	0.64 $\pm$ 0.06	> 1000 (20.8%) ^{a)}
7	0.74 $\pm$ 0.05	31.1 $\pm$ 4.42
12	0.63 $\pm$ 0.11	300 $\pm$ 29.6
13	0.026 $\pm$ 0.004	100 $\pm$ 9.37
14	0.016 $\pm$ 0.003	2.55 $\pm$ 0.63

^{a)} Percent inhibition at 1000 μM with S.D. less than 10%.

IC_{50} values are means $\pm$ S.D. of three experiments. [^3H]PCP binding was initiated by addition of honeybee head membranes, and was terminated after 40 sec incubation. Chemical structures are listed in Fig. 1.

Fig. 5 Effect of replacing the 3-pyridylmethyl group with benzyl group on the [^3H]PCP binding in the absence (left and center) and presence (right) of 100 μM carbachol in the Torpedo.

The binding reaction was performed under the condition of 60 sec incubation. Symbols represent mean of three experiments with S.D. less than 10%. Numbers on graphs refer to compounds listed in Fig. 1.

a certain potency as inhibitor of [^3H] α -BGT binding. Benzylamines having *N,N*-dimethyl (**12**) and piperidyl (**13**) substituents were much more effective on the [^3H]PCP binding than the [^3H] α -BGT binding, whereas PCP (**14**) showed relatively high potency to the [^3H] α -BGT binding and the highest potency to the [^3H]PCP binding (Table 2).

6. Binding Properties of 3-Pyridylmethylamines and PCP to the Honeybee nAChR/Ion Channel Complex

Binding properties of *N,N*-di-*n*-butyl (**6**) and piperidyl (**7**) analogs of 3-pyridylmethylamine as well as PCP (**14**) were estimated by pseudo-Scatchard analysis using the data for titration of inhibitor on the binding of 5 or 10 nM [^3H]PCP with 40 sec and 30 min incubation (Table 3). In the condition of 40 sec incubation, compounds **6**, **7** and **14** demonstrated a single binding site in each case, as indicated by the Hill coefficients of 1.01 to 1.04. The titration of unlabeled PCP on the binding of 10 nM [^3H]PCP under the 40 sec incubation gave the

Fig. 6 Scatchard plots for 5 nM [^3H]PCP binding to the membranes from the honeybee heads in the absence (●) and presence (○) of 0.6 μM 3-pyridylmethyl-*N,N*-di-*n*-butylamine (**6**) with 40 sec incubation. Binding parameters are as follows: K_D 19.6 $\pm$ 0.52 nM, B_{max} 763 $\pm$ 11.6 fmol/mg protein and Hill coefficient (n_H) 1.06 $\pm$ 0.03 in the absence of compound **6**, while K_D 40.0 $\pm$ 1.98 nM, B_{max} 759 $\pm$ 25.4 fmol/mg protein and Hill coefficient (n_H) 1.03 $\pm$ 0.05 in the presence of compound **6**. Values are means $\pm$ S.D. of three experiments. * Significant difference between control and treatment ($p < 0.01$).

same result as 5 nM [^3H]PCP (data not shown). On the other hand, 30 min incubation experiment gave Hill coefficients less than 1.00 for compounds **7** and **14**, suggesting that there are two binding sites with high and low affinities. Their IC_{50} values as inhibitors of [^3H] α -BGT binding were similar as those for the low affinity site (designated as site 2) of [^3H]PCP binding under the 30 min incubation. In contrast, compound **6** only displayed a single site with a Hill coefficient of 1.01 even under 30 min incubation, and did not affect the ACh site as indicated by the inhibition of [^3H] α -BGT binding. Although IC_{50} values of the three compounds (**6**, **7** and **14**) under the 40 sec incubation were not identical to those for the high affinity site (designated as site 1) under 30 min incubation, the order and ratio of potency among the three compounds were comparable between two conditions.

7. Effect of 3-Pyridylmethyl-*N,N*-di-*n*-butylamine on K_D and B_{max} Values of [^3H]PCP Binding to the Honeybee nAChR/Ion Channel Complex

Since 40 sec incubation only allows to label the high affinity binding site of [^3H]PCP, the effect of 3-pyridylmethyl-*N,N*-di-*n*-butylamine (**6**) on K_D and B_{max} values of [^3H]PCP binding was investigated under the 40 sec incubation to analyze the high affinity binding site. Scatchard plots for 5 nM [^3H]PCP binding in the absence and presence of 0.6 μM compound **6** showed the convergence of two lines to a point on the X axis. The K_D value in the presence of compound **6** was significantly different from control, while B_{max} values in both conditions were identical. Hill coefficient indicated a single site action for both cases (Fig. 6).

DISCUSSION

When the *Torpedo* nAChR is in the activated status, increasing the chain length of *N,N*-dialkyl substituents of 3-pyridylmethylamine from dimethyl to di-*n*-butyl (**2**, **4**–**6**) enhances the potency as inhibitors of [^3H]PCP binding, whereas it reduces the agonistic potency based on the inhibition of [^3H] α -BGT binding and the stimu-

Table 3 Binding properties of 3-pyridylmethylamines and PCP on the [^3H]PCP and [^3H] α -BGT bindings to the membranes from the honeybee heads.

Compound no.	[³ H]PCP binding					[³ H] α-BGT binding
	5 nM, ^{a)} 40 sec ^{b)}		10 nM, ^{a)} 30 min ^{b)}			
	IC ₅₀ , μM	n _H ^{c)}	IC ₅₀ (site 1), μM	n _H ^{c)}	IC ₅₀ (site 2), μM	IC ₅₀ , μM
6	0.64±0.06	1.03±0.04	2.66±0.38	1.01±0.02	ND ^{d)}	> 1000 (20.8%) ^{e)}
7	0.74±0.05	1.01±0.02	2.96±1.17	0.93±0.03	13.7±2.40	31.1±4.42
14	0.016±0.003	1.04±0.03	0.042±0.009	0.84±0.03	1.79±0.67	2.55±0.63

^{a)} Concentration of [^3H]PCP. ^{b)} Incubation period. ^{c)} Hill coefficient. ^{d)} Not detected. ^{e)} Percent inhibition at 1000 μM . Values are means $\pm$ S.D. of three experiments. Chemical structures are listed in Fig. 1.

lation of [^3H]PCP binding. Scatchard analysis of [^3H]PCP binding stimulated by carbachol reveals that 3-pyridylmethyl-*N,N*-di-*n*-butylamine (**6**) interacts with a NCB site located in the ion channel of *Torpedo* nAChR in the activated status. The same interaction is also seen for trimethaphan³⁾ and physostigmine.^{13,14)} Among three pyridyl isomers of pyridylmethyl-*N,N*-dimethylamine, a 3-pyridyl isomer (**2**) shows the highest agonistic effect, although 2- and 4-isomers (**1**, **3**) are also effective. In addition, tetramethylammonium compound (**8**) shows an agonistic effect. The pyridylmethyl-*N,N*-dimethylamines with highly basic amino nitrogen atom mostly ionize by protonation at physiological pH.⁹⁻¹¹⁾ These suggest that although the presence of 3-pyridylmethyl moiety is preferred, the ammonium head with low alkyl substituents like methyl is important to be agonistic at the *Torpedo* nAChR.

As previously reported by Bakry *et al.*,¹⁵⁾ we confirm that increasing the chain length of a homologous series of symmetrically substituted tetraalkylammonium compounds from tetramethyl to tetra-*n*-butyl (**8-11**) reduces the affinity to the ACh site, while it enhances the affinity for the channel site of *Torpedo* nAChR. It is interesting to note that this relationship between chemical structure and binding site discrimination of tetraalkylammonium compounds is similar to those of 3-pyridylmethylamines.

Effect of replacing the 3-pyridylmethyl group with benzyl group on the potency of compound to the *Torpedo* nAChR suggests that the ACh site prefers 3-pyridylmethyl group over benzyl group. On the other hand, benzyl group is suitable for the channel site as shown in inhibition of [^3H]PCP binding when receptor is in the activated status. Introduction of a piperidyl group to the 3-pyridylmethyl moiety (**7**) gives a suitable agonistic effect and weak channel blockade effect when the ion channel is opened. In contrast, *N*-benzylpiperidine (**13**) shows very weak effect on the activation of [^3H]PCP binding and a certain inhibition of [^3H]PCP binding in the opened status of ion channel. Furthermore, PCP (**14**) with a cyclohexyl moiety [*N*-(1-phenylcyclohexyl)piperidine] shows a complete channel blockade effect. Such structural modification influences the binding site recognition between the ACh and NCB sites in the *Torpedo* nAChR.

Unlike binding to the *Torpedo* nAChR, [^3H]PCP binding is not activated by cholinergic agonists in insect nAChRs.^{7,8,16)} In insect, PCP binds both to the NCB and ACh sites. However, combination of [^3H]PCP and [^3H] α -BGT binding assays is available to estimate the interaction of ligands with the ACh and NCB sites of insect nAChR.⁸⁾ As for the effects of tested compounds on the binding of two radioligands in the insect nAChR, potencies of compounds as inhibitors of [^3H] α -BGT binding does not coincide with those as inhibitors of [^3H]PCP binding. This suggests that [^3H]PCP binds

to different site from [^3H] α -BGT. Substituent effect on potency of compound as inhibitors of two radioligands binding to insect nAChR is similar to that to *Torpedo* nAChR, *i.e.* increasing the chain length of *N,N*-dialkyl substituents of 3-pyridylmethylamine from dimethyl to di-*n*-butyl (**2**, **4-6**) enhances the potency as inhibitor of [^3H]PCP binding, whereas it reduces the affinity to the ACh site in insect nAChR. Furthermore, compounds having benzylamine and piperidyl moieties such as PCP are preferred over compounds having 3-pyridylmethylamine moiety in inhibition of [^3H]PCP binding in insect, a relationship similar to that in *Torpedo*.

Our experiment shows that bindings of PCP and 3-pyridylmethylamines to the insect nAChR/ion channel complex can be affected by the incubation period. The IC_{50} values of compounds **6**, **7** and **14** for the low affinity site (designated as site 2) of the [^3H]PCP binding predict their affinities to the ACh site as indicated by the inhibition of [^3H] α -BGT binding. We propose that PCP binds both to the NCB site with high affinity and the ACh site with low affinity in the insect nAChR.⁸⁾ This suggests that the high and low affinity sites for *N*-(3-pyridylmethyl)piperidine (**7**) on [^3H]PCP binding correspond to the NCB and ACh sites of insect nAChR, respectively. On the other hand, 3-pyridylmethyl-*N,N*-di-*n*-butylamine (**6**) does not affect the ACh site, although it selectively inhibits [^3H]PCP binding. Scatchard analysis of [^3H]PCP binding to the insect nAChR suggests that compound **6** interacts with high affinity site for PCP, which is displaceable site by NCBs such as *N*-[1-(2-thienyl)cyclohexyl]piperidine (thienyl analog of PCP; TCP), mecamlamine, chlorpromazine and ketamine *etc.*, but not by α -BGT.⁸⁾ Accordingly, the binding site for 3-pyridylmethyl-*N,N*-di-*n*-butylamine (**6**) coincides with the NCB site located in the ion channel of insect nAChR.

The conformational analyses of the nicotinic ligands reveal the molecular feature to interact with the ACh site: the presence of an electron-donating group which makes hydrogen bonding to the site at 5.9 Å apart from positively charged center of the ligand.¹⁷⁾ Thus 3-pyridylmethylamine moiety satisfies this condition.¹²⁾ The longer alkyl or piperidyl substituents of 3-pyridylmethylamine and tetraalkylammonium compound as well as benzylamine prefer the NCB site over the ACh site. The nAChR subunit has four transmembrane segments (referred to as M1, M2, M3 and M4) based on the hydrophobicity profile of the primary amino acid sequence.¹⁸⁻²⁰⁾ It is known that the hydrophobic M2 segment is a component of the ion channel and contributes to the NCB site.^{18,19)} These SARs can be rationalized as an interaction of apolar moiety in relation to its steric features with the hydrophobic M2 segment of ion channel.

In summary, our study suggests that the systematic changes within a series of analogs convert the binding site of ligand from the ACh to NCB sites of nAChRs in both *Torpedo* and insect. These information on SARs will facilitate development of a possible probe for the NCB site of insect nAChR/ion channel complex.

ACKNOWLEDGMENTS

We wish to express our gratitude to Professor John E. Casida and Dr. Jian Huang of the University of California at Berkeley and Professor Mohyee E. Eldefrawi of the University of Maryland at Baltimore for valuable discussions. This study was also facilitated by colleagues Tadachika Ota and Yoko Yasui of the Radioisotope Center of the Tokyo University of Agriculture.

REFERENCES

- 1) M. E. Eldefrawi, A. T. Eldefrawi, R. S. Aronstam, M. A. Maleque, J. E. Warnick & E. X. Albuquerque: *Proc. Natl. Acad. Sci. U.S.A.* **77**, 7458 (1980)
- 2) R. S. Aronstam, A. T. Eldefrawi, I. N. Pessah, J. W. Daly, E. X. Albuquerque & M. E. Eldefrawi: *J. Biol. Chem.* **256**, 2843 (1981)
- 3) M. Tomizawa, H. Otsuka, T. Miyamoto & I. Yamamoto: *J. Pesticide Sci.* **20**, 49 (1995)
- 4) E. X. Albuquerque, A. T. Eldefrawi & M. E. Eldefrawi: "Snake Venoms," ed. by C.-Y. Lee, Springer-Verlag, Berlin, pp. 377-402, 1979
- 5) D. B. Sattelle, I. D. Harrow, B. Hue, M. Pelhate, J. I. Gepner & L. M. Hall: *J. Exp. Biol.* **107**, 473 (1983)
- 6) S. C. R. Lummis & D. B. Sattelle: *Comp. Biochem. Physiol.* **80C**, 75 (1985)
- 7) D. B. Sattelle, B. Hue, M. Pelhate, S. M. Sherby, A. T. Eldefrawi & M. E. Eldefrawi: *J. Insect Physiol.* **31**, 917 (1985)
- 8) M. Tomizawa, H. Otsuka, T. Miyamoto, M. E. Eldefrawi & I. Yamamoto: *J. Pesticide Sci.* **20**, 57 (1995)
- 9) I. Yamamoto, H. Kamimura, R. Yamamoto, S. Sakai & M. Goda: *Agric. Biol. Chem.* **26**, 709 (1962)
- 10) M. Tomizawa & I. Yamamoto: *J. Pesticide Sci.* **17**, 231 (1992)
- 11) M. Tomizawa: *J. Pesticide Sci.* **19**, S229 (1994)
- 12) I. Yamamoto, G. Yabuta, M. Tomizawa, T. Saito, T. Miyamoto & S. Kagabu: *J. Pesticide Sci.* **20**, 33 (1995)
- 13) S. M. Sherby, A. T. Eldefrawi, E. X. Albuquerque & M. E. Eldefrawi: *Mol. Pharmacol.* **27**, 343 (1985)
- 14) A. Schratzenholz, J. Godovac-Zimmermann, H.-J. Schäfer, E. X. Albuquerque & A. Maelicke: *Eur. J. Biochem.* **216**, 671 (1993)
- 15) N. M. Bakry, A. T. Eldefrawi, M. E. Eldefrawi & W. F. Riker, Jr.: *Mol. Pharmacol.* **22**, 63 (1982)
- 16) A. T. Eldefrawi, N. Shaker & M. E. Eldefrawi: "Neuropharmacology of Insect (Ciba Foundation Symposium 88)," ed. by D. Evered, M. O'Connor & J. Whelan, Pitman, London, pp. 137-152, 1982
- 17) W. H. Beers & E. Reich: *Nature* **228**, 917 (1970)
- 18) J.-L. Galzi, F. Revah, A. Bessis & J.-P. Changeux: *Annu. Rev. Pharmacol.* **31**, 37 (1991)
- 19) A. Devillers-Thiéry, J.-L. Galzi, J. L. Eisélé, S. Bertrand, D. Bertrand & J.-P. Changeux: *J. Membrane Biol.* **136**, 97 (1993)
- 20) E. D. Gundelfinger & N. Hess: *Biochim. Biophys. Acta* **1137**, 299 (1992)

要 約

3-ピリジルメチルアミンのアミノ窒素原子のジアルキル置換基はシビレエイおよび昆虫ニコチンクレセプターの α -ブンガロトキシン部位とイオンチャンネル部位とを識別する

富澤元博, 松尾花子, 宮本 徹, 山本 出
シビレエイのニコチンクレセプターにおいて, 3-ピリジルメチルアミンのアミノ窒素原子上のジアルキル置換基の長さをジメチルからジブチルまで増加させるに従い, そのアゴニスト作用が漸進的に減少, またはみられなくなった。ところがイオンチャンネルの開口時では置換基の長さに依存してイオンチャンネル部位への親和性が漸進的に増大した。このような置換基の長さと結合部位の識別との関係は, テトラアルキルアンモニウム化合物のそれにも同様の傾向を認めた。さらに昆虫のニコチンクレセプターにおいても, 3-ピリジルメチルアミンの窒素原子上のジアルキル置換基の長さに比例して α -ブンガロトキシン部位, すなわちアセチルコリン部位への作用は減少したが, これとは対照的に高親和性のフェンサイクリジン部位, すなわちイオンチャンネル部位への親和性は漸進的に増大した。