Note

Comparison of the Binding Activities of Chloronicotinyl Insecticides toward the Nicotinic Acetylcholine Receptors from Rats and Houseflies

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INTRODUCTION

Chloronicotinyl insecticides such as imidacloprid, acetamiprid and nitenpyram are a class of novel potent insecticides developed by Japanese and German companies in the late 1980s. These insecticides have the 6-chloro-3-pyridylmethyl moiety as a common structure and the remaining functional groups can be either cyclic or acyclic. This class of compounds is characterized by broad spectra, good systemic properties, low toxicity to mammals and aquatic life, and moderate stability in agricultural fields.¹⁻⁶⁾

This class of insecticides acts at the nicotinic acetylcholine receptor (nAChR) of insects by selectively perturbing the neuronal signal transduction.^{7,8)} Radiolabelled α -bungarotoxin (α -BGTX), which binds antagonistically to acetylcholine binding sites of the nAChR, has been widely used in competitive receptor-binding assays to evaluate the bioactivity of these compounds.^{7,9-11)} Recently, [³H]imidacloprid was also used in this receptor-binding assay to clarify the binding mode of this class of insecticides.¹²⁻¹⁷⁾ A good correlation between the binding activities measured using [³H] α -BGTX and [³H]imidacloprid has been reported.¹⁸⁾ However, the exact binding modes of these insecticides have not yet been fully elucidated.

There are two types of nAChRs which can be distinguished from each other by the response of mammalian brain tissue to the binding of α -BGTX and *l*-nicotine.¹⁹⁾ There are several reports dealing with the binding activity of chloronicotinyl insecticides to mammalian nAChR in terms of competition of [³H]*l*-nicotine,^{11,20)} however, no information on binding assays using α -BGTX has been found. In this study, we

measured and compared the binding activities of chloronicotinyl compounds including imidacloprid, nitenpyram and acetamiprid to the α -BGTX-sensitive nAChR prepared from Wister rat brain and housefly (*Musca domestica* L.) heads.

MATERIALS AND METHODS

1. Test Chemicals

The tested compounds are listed in Tables 1 and 2. The following compounds were kindly provided by several companies: Compounds **1-19**, Nihon Bayer Agrochem Co. (Ibaraki, Japan); compounds **20-31**, Takeda Chemical Industries, Ltd. (Osaka, Japan); compound **32**, Nippon Soda Co., Ltd. (Tokyo, Japan). α -BGTX was purchased from Sigma Chemical Co. (St. Louis, MO, USA). [¹²⁵I] α -BGTX (74 TBq/mmol) was purchased from Amersham International (Buckinghamshire, UK). BCA protein assay reagents were purchased from Pierce (Rockford, IL, USA).

2. nAChR Preparation

2.1 Rat brain

Crude nAChR was prepared using rat brains by the conventional method.²¹⁾ Male Wister rats (200 g) were decapitated and the brains were quickly excised. The brains were homogenized (10% wt/vol) in saline containing 0.32 M sucrose, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride, and 0.1% (wt/vol) sodium azide. The homogenates were then centrifuged at 1000×*g* for 10 min, and the supernatant was decanted and retained on ice. The pellet was suspended in 0.32 M sucrose (5 ml/g original wet weight) and centrifuged at 1000×*g* for 10 min. The combined supernatants were centrifuged at 12,000×*g* for 30 min to give a pellet which was resuspended in binding assay buffer (pH 7.4) containing 50 mM potassium phosphate and protease inhibitors as described above (2.5 ml/g of original wet weight of the rat brain). The protein concentration was determined by BCA protein assay reagents using BSA as a standard.

2.2 Housefly heads

A housefly head membrane fraction was prepared according to our previously described method.²²⁾

3. Binding Assays

Binding assays were done according to the method previously described.²²⁾ Aliquots of rat-nAChR preparations (ca. 5 μ g protein) were incubated with [¹²⁵I] α -BGTX (0.5 nM) at 25°C for 60 min in a microplate. Similar assays were done for housefly-nAChR preparations (ca. 250 μ g protein) by incubating with test chemicals and [¹²⁵I] α -BGTX (0.2 nM). Test chemicals were used at appropriate concentrations (ca. 1×10^{-2} – 10^{-9} M) in the corresponding binding assay buffer (200 μ l). The reaction was terminated by rapid filtration through a Unifilter GF/B (Packard Instrument Co., Meriden,

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Table 1 Binding activities of imidacloprid and related compounds.

No.	Substituents				log(1/ K_i)	
	X	Y	Z	n	Rat	Housefly ^a
1	3-Pyridylmethyl	CHNO ₂	NH	2	3.47	7.30
2	6-Cl-3-pyridylmethyl	CHNO ₂	NH	2	4.15	7.49
3	6-Me-3-pyridylmethyl	CHNO ₂	NH	2	3.07	6.72
4	6-Cl-3-pyridyl	CHNO ₂	NH	2	<2.00	4.47
5	2-(6-Cl-3-pyridyl)ethyl	CHNO ₂	NH	2	3.25	4.71
6	2-Pyridylmethyl	CHNO ₂	NH	2	<2.00	4.84
7	4-Pyridylmethyl	CHNO ₂	NH	2	<2.00	3.14
8	Benzyl	CHNO ₂	NH	2	<2.00	5.49
9	4-Cl-benzyl	CHNO ₂	NH	2	3.08	5.30
10	5-(2-Cl-thiazolyl)methyl	CHNO ₂	NH	2	4.24	6.97
11	3-Pyridylmethyl	NNO ₂	NH	2	<2.00	5.35
12 ^b	6-Cl-3-pyridylmethyl	NNO ₂	NH	2	4.08	6.00
13	6-Cl-3-pyridylmethyl	NNO ₂	NMe	2	2.42	3.83
14	6-Cl-3-pyridylmethyl	NCN	NH	2	<2.00	5.09
15	6-Cl-3-pyridylmethyl	CHCN	NH	2	2.49	5.11
16	6-Cl-3-pyridylmethyl	CHNO ₂	CH ₂	2	3.00	7.11
17	6-Cl-3-pyridylmethyl	CHNO ₂	O	2	2.91	6.40
18	6-Cl-3-pyridylmethyl	CHNO ₂	S	2	2.92	7.00
19	6-Cl-3-pyridylmethyl	CHNO ₂	NH	3	3.02	7.70

^a Taken from ref 22. ^b Imidacloprid.

Table 2 Binding activities of nitenpyram and related compounds.

No.	Substituents			log(1/ K_i)	
	R ₁	Y	R ₂	Rat	Housefly
20	H	CHNO ₂	NH ₂	2.42	3.71
21	H	CHNO ₂	NHN(CH ₃) ₂	<2.00	3.57
22	Me	CHNO ₂	NHMe	2.75	5.48
23	Me	CHNO ₂	NHEt	<2.00	3.39
24 ^a	Et	CHNO ₂	NHMe	<2.00	5.17
25	Et	CHNO ₂	NHEt	<2.00	3.46
26	Et	CHNO ₂	N(CH ₂ CH ₃) ₂	2.45	3.27
27	Et	CHNO ₂	NH(cyclopropyl)	2.45	3.62
28	Et	CHNO ₂		<2.00	3.24
29	CH ₂ CH ₂ F	CHNO ₂	NH ₂	2.85	6.24
30	Pr	CHNO ₂	NH ₂	3.99	5.96
31	<i>i</i> -Pr	CHNO ₂	NHMe	2.97	4.14
32 ^b	Me	NCN	Me	2.75	5.31

^a Nitenpyram. ^b Acetamiprid.

CT, USA) that had been treated with 0.1% polyethylenimine to prevent the nonspecific binding of [¹²⁵I] α -BGTX to the filter.²³⁾ The filters were rinsed three times with the binding buffer, followed by methanol, and then dried. Total radioactivity captured in the filter was measured as counts per

minute (cpm) using a Topcount (Packard) instrument, after addition of Microscinti-O (30 μ l; Packard). Specific binding was defined as the difference in radioactivities measured in the absence (10,000–15,000 cpm) and presence (2000–3000 cpm) of 10 μ M unlabelled α -BGTX. The saturation curve for the

nAChRs was obtained by using only [125 I] α -BGTX at various concentrations. All tests were done in triplicate, and the mean values of the radioactivity were used for further calculations.

4. Data Calculations

The molar concentrations required for 50% inhibition of the specific binding of [125 I] α -BGTX (IC_{50}) were determined by nonlinear regression analysis using PRISM (Graphpad Software Inc., San Diego, CA, USA). The binding activity of test chemicals was evaluated by calculating K_i using the following equation:²⁴⁾

$$K_i = IC_{50} / (1 + [L] / K_d),$$

where $[L]$ is the concentration of the radiolabelled ligand and K_d is the dissociation constant. PRISM was used to determine K_d values for rat (0.69 nM) and housefly preparations (0.058 nM) through nonlinear regression analysis of [125 I] α -BGTX saturation curves. The $\log(1/K_i)$ values, which are listed in Tables 1 and 2, were taken as the index of the binding activity. The binding assay was repeated for several compounds to assess the reproducibility of the $\log(1/K_i)$ within an error of ± 0.3 .

RESULTS AND DISCUSSION

We were able to determine $\log(1/K_i)$ values with the housefly preparation for all of the compounds tested, whereas $\log(1/K_i)$ values were determinable for only 21 compounds with the rat preparation (Tables 1 and 2). The $\log(1/K_i)$ values for housefly preparation varied from 3.1 to 7.7, and the values for rat preparation ranged from 2.4 to 4.3 or lower. All of the tested compounds showed higher binding activity to housefly nAChR than to rat brain nAChR. This result demonstrates that the binding to α -BGTX-sensitive insect-nAChR is dependent much more than the mammal-nAChR upon chemical structure of this class of compounds. The present results with the rat nAChR were in agreement with the binding activity of imidacloprid (determined using [3 H]*I*-nicotine with rat nAChR¹¹⁾ and of compounds **1**–**19** (using [3 H]*I*-nicotine with mouse nAChR²⁰⁾). Consequently, the high selective toxicity of this class of compounds to insects might be due to their high binding affinity, representatively demonstrated with housefly nAChR, and low binding affinity to rat nAChR.

In the rat-nAChR binding assay, imidacloprid (**12**) was more active than nitenpyram (**24**) and acetamiprid (**32**) (Tables 1 and 2). This is consistent with the rating of their toxicity to mice and rats.⁴⁾ Nitenpyram (**24**) showed the lowest toxicity to mice and rats.⁴⁾ Its binding activity towards rat nAChR was not determinable in terms of $\log(1/K_i)$. Cyclic compounds **2** and **10**, and the acyclic compound **30** showed similar binding activity to imidacloprid (**12**) in rat brain preparation. Because of the small variations in the activity toward the rat nAChR, it was difficult to quantitatively draw any substructures required for binding.

Recently, insect nAChR was purified by affinity chromatography using an imidacloprid derivative.²⁵⁾ The structure of *Torpedo* nAChR has been shown by electron microscopy²⁶⁾ and NMR²⁷⁾ techniques. Both of these studies on nAChR, as well as our structural analyses of the insecticides, aid in revealing the mode of selectivity and structural requirements necessary for a high affinity to the insect nAChR. We predicted the binding mode of imidacloprid and its cyclic derivatives by a technique of three-dimensional quantitative structure analysis using the binding data determined with the housefly nAChR.²²⁾ Analyses are currently under way for acyclic chloronicotinyl insecticides.

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要 約

ラットとイエバエのニコチン性アセチルコリン受容体に対するクロロニコチニル殺虫剤の結合活性の比較

岡澤敦司, 中川好秋, 赤松美紀, 上野民夫, 西村勁一郎
イミダクロプリド, アセタミプリド, ニテンピラムおよびそれらの類縁化合物のラットとイエバエのニコチン性アセチルコリン受容体に対する結合活性を, $[^{125}\text{I}]\alpha\text{-bungarotoxin}$ を用いて測定した。用いた濃度において, すべての化合物がイエバエの受容体に対する結合活性を有したが, ラットの受容体に対しては 21 化合物しか活性を示さなかった。また, ラットの受容体に対する活性値はイエバエの受容体に対する活性値の $1/10 \sim 1/10,000$ であった。なお, これらの殺虫剤のラットに対する毒性の強さは, ラットの受容体に対する結合活性値の高さに対応していた。本実験の結果より, クロロニコチニル殺虫剤の選択性は, その受容体への結合活性の差によって説明できることが示された。