J. Pestic. Sci., **29**(3), 177–183 (2004)

Review

Neonicotinoids and Derivatives: Effects in Mammalian Cells and Mice

Motohiro TOMIZAWA*

Environmental Chemistry and Toxicology Laboratory, Department of Environmental Science, Policy and Management, University of California, Berkeley, California 94720–3112, USA

(Received April 9, 2004)

Neonicotinoids are the only major new class of insecticides introduced in the past three decades. They act as selective agonists at the insect nicotinic acetylcholine receptor and are therefore highly toxic towards important insect pests but relatively safe to mammals. However, the excellent selective toxicity may not be evident with their metabolites or analogous compounds. The aim of this paper is to consider the effects of neonicotinoids and derivatives in mammalian cells and mice involving up-regulation of nicotinic receptor levels and activation of the intracellular signal integration cascade elicited by chronic or sustained exposure and analgesic and toxic effects in mice.

Keywords: analgesia, extracellular signal-regulated kinase cascade, neonicotinoids, nicotinic receptors, toxicity, up-regulation.

1. INTRODUCTION

The major insecticides are neurotoxicants acting on the voltage-dependent sodium channel, the chloride channel of the γ-aminobutyric acid receptor, and the cholinergic system as inhibitors of acetylcholinesterase or agonists at the nicotinic acetylcholine receptor (nAChR). Selective toxicity involving low hazard for mammals and high potency to pests is an essential requirement for safe and effective insecticides.[1,2] Neonicotinoids are the most important new class of synthetic insecticides of the past three decades and are increasingly used in crop protection and flea control in companion animals.[3–7] The excellent selective toxicity of the neonicotinoids is conferred in large part by differential sensitivity for insect versus mammalian nAChRs. However, this observation is based on only the parent insecticide. The selectivity profile of neonicotinoids is not shared with desnitro or descyano metabolites (in animals and/or plants) and derivatives which exhibit high toxicity to mice and high affinity and/or agonist potency to mammalian nAChRs equal to or greater than that of nicotine.[7] In order to better understand the actions of neonicotinoid insecticides in mammals, this review discusses several studies on the actions of neonicotinoids and derivatives in mammalian cells and mice. Specific aspects focused on here are: **1**) up-regulation of receptor numbers and activation of intracellular signaling pathways elicited by chronic and sustained treatments in mammalian cells; and **2**) analgesic activity and adverse toxic effects in mice associated with their action at the target site.

2. STRUCTURAL FEATURES OF NEONICOTINOIDS AND DERIVATIVES

The neonicotinoids [exemplified here by imidacloprid (IMI) and thiacloprid] and nicotinoids [nicotine and epibatidine (a toxin isolated from the skin of Ecuadoran frog)] have some common structural features but are distinctly different in being nonprotonated and predominantly protonated, respectively, under physiological conditions. Desnitro-IMI (DNIMI) and descyano-thiacloprid are mostly protonated at physiological pH and prefer mammalian versus insect nAChRs.[8] On this basis, these neonicotinoid *N*-unsubstituted imines have nicotinoid-type of action (Fig. 1). The unique aspect of the neonicotinoid structure is a *N*-nitroimine or *N*-cyanoimine substituent. This electronegative pharmacophore plays the crucial role in the high affinity and selectivity for the insect nAChRs.[8,9] Other structural features also affect the selectivity for insect versus mammalian nAChRs. Introduction of azido or amino at the 5-position of the 6-chloropyridin-3-yl moiety of neonicotinoids and epibatidine reduces the potency for the insect nAChR but not for the mammalian receptors.[10,11]

* To whom correspondence should be addressed.
E-mail: tomizawa@nature.berkeley.edu

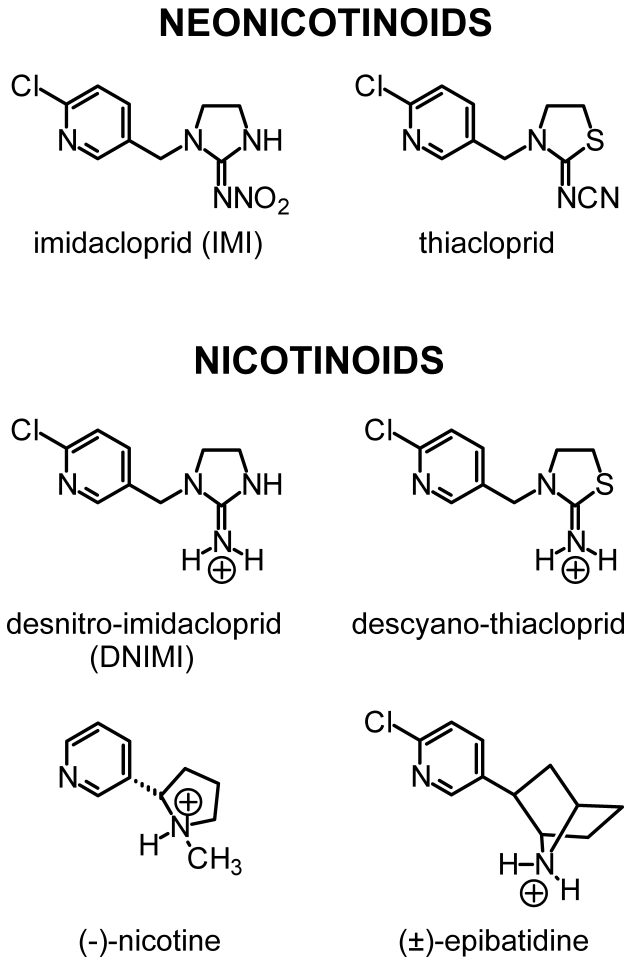

Fig. 1. Structures of neonicotinoids [imidacloprid (IMI) and thiacloprid] selective for the insect nAChR and nicotinoids [desnitro-IMI (DNIMI), descyano-thiacloprid, (−)-nicotine, and (±)-epibatidine] selective for the mammalian receptor. The neonicotinoids with an electronegative pharmacophore (=NNO_2 or =NCN) are not protonated, whereas the nicotinoids with a basic nitrogen atom are protonated at physiological condition.

3. VERTEBRATE NICOTINIC RECEPTORS

3.1. Nicotinic receptors

The vertebrate nAChR consists of diverse subtypes assembled in combinations from ten α, four β, γ, δ, and ε subunits. The skeletal muscle or *Torpedo* electric organ subtype is made up of two $\alpha 1$ subunits and one each of $\beta 1$, γ, and δ (or ε in adult muscle) subunits. Neuronal nAChR subtypes expressed in vertebrate brain and ganglia are assembled in combinations of $\alpha 2$–10 and $\beta 2$–4 and are pharmacologically classified into two groups based on sensitivity to α-bungarotoxin (α-BGT). The $\alpha 2$–6 and $\beta 2$–4 subunits are involved in assembling the α-BGT-insensitive subtypes, while the $\alpha 7$–10 subunits are responsible for α-BGT-sensitive receptors. Of these, the most abundant subtypes in vertebrate brain are $\alpha 4\beta 2$ and $\alpha 7$. The $\alpha 4\beta 2$ subtype consists of two $\alpha 4$ and three $\beta 2$ subunits (heteropentameric) and the $\alpha 7$ subtype is considered to be a homopentameric structure.[12)]

3.2. Subtype selectivity

The agonist-binding site is localized at the interface region between subunits. Specific subunit combinations confer differences in sensitivity to acetylcholine and/or pharmacological profiles among the nAChR subtypes. The ligand-binding site in all subtypes consists of a conserved core of aromatic amino acid residues,[13–15)] and variable residues neighboring the conserved aromatic residues are considered to confer the individual pharmacological properties of each subtype.[12)] The mammalian nAChR is the target for potential therapeutic agents for analgesia, neurodegenerative diseases, cognitive dysfunction, schizophrenia, depression, and anxiety.[16)] An important aspect of nicotinic drug development is the quest for high subtype-selectivity.[16,17)]

3.3. Actions of neonicotinoids and derivatives

Neonicotinoids have little or no effect on any of the vertebrate muscle nAChR subtype $\alpha 1\gamma\alpha 1\delta\beta 1$ and neuronal subtypes including $\alpha 3\beta 2$(and/or $\beta 4$)$\alpha 5$, $\alpha 4\beta 2$, and $\alpha 7$. However, minor structural modifications confer differential selectivity in vertebrate nAChRs: i.e., desnitro and descyano metabolites of neonicotinoids display high potency particularly to the $\alpha 4\beta 2$ subtype equal to or greater than that of nicotine (but they are deactivation products for the insect receptor); nitromethylene analogues of neonicotinoids display higher or comparable affinity to that of nicotine at the $\alpha 7$ or $\alpha 3\beta 2\beta 4\alpha 5$ subtype, respectively. Toxicological evaluations of insecticide safety should also consider the subtype level.[7,8,18)]

4. EFFECTS IN MAMMALIAN CELLS

4.1. Up-regulation of $\alpha 4\beta 2$ nicotinic receptor levels elicited by neonicotinoids and derivatives

Chronic nicotine treatment *in vivo* elicits up-regulation of brain nAChR levels (increase in numbers) based on radioligand binding[19–22)] and autoradiographic analysis.[23–25)] Studies on up-regulation of neuronal nAChRs are expedited by *in vitro* systems such as cell lines transfected with $\alpha 4$ and $\beta 2$ subunits or primary cultured neurons.[26–29)] The hypothesis to be tested was that neonicotinoids and their *N*-unsubstituted imine metabolites also up-regulate the nAChRs.

Chronic exposure (3 days) of M10 cells to the neonicotinoid insecticides (IMI and thiacloprid) and their *N*-unsubstituted imines (DNIMI and descyano-thiacloprid), as with nicotine and epibatidine, elicits an approximately 5- to 8-fold up-regulation of $\alpha 4\beta 2$ nAChR levels.[30)] Importantly, the EC_{50} values of IMI and thiacloprid are 25- to 92-fold higher than that of nicotine (Table 1). However, DNIMI and descyano-thiacloprid are of similar potency to that of nicotine.[30)] The ligand binding site is not altered in affinity for DNIMI on chronic DNIMI treatment[30)] consistent with the findings on nicotine.[26,27)] In contrast, the $\alpha 4\beta 2$ receptor is functionally

Table 1. Potencies of neonicotinoid insecticides, their *N*-unsubstituted imine metabolites, and standards for up-regulation of $\alpha4\beta2$ nAChR levels and as inhibitors of [^{3}H]nicotine binding[a]

Compound	Up-regulation EC_{50} (nM)[b]	[^{3}H]Nicotine binding IC_{50} (nM)[c]
Neonicotinoids		
IMI	~70,000	2,600
Thiacloprid	19,000	900
***N*-Unsubstituted imine metabolites**		
DNIMI	870	8.2
Descyano-thiacloprid	500	4.4
Standards		
(−)-Nicotine	760	7.0
(±)-Epibatidine	10	0.04

[a] Data from Ref. 30. [b] Concentration for half-maximal up-regulation of $\alpha4\beta2$ nAChR numbers expressed in mouse fibroblast M10 cells evoked by chronic exposure to test compound for 3 days. [c] Binding affinity to $\alpha4\beta2$ nAChR immunoisolated by monoclonal antibody 299 from M10 cells.

up-regulated by chronic nicotine exposure in K-177 cell line.[31] The up-regulation of $\alpha4\beta2$ nAChRs by neonicotinoid derivatives is initiated by receptor-ligand interaction based on the excellent correlation of EC_{50} values with IC_{50}s for receptor binding ($r^2=0.99$).[30] Nicotine up-regulates the receptor post-translationally since the levels of $\alpha4$ and $\beta2$ mRNAs are unaffected in brain, neurons, and transfected cells.[22,24,26,27,32] The up-regulation reflects properties of the receptor protein, rather than an adaptive mechanism to compensate for the loss of neuronal function. These findings on nicotine-induced up-regulation are probably also applicable to the neonicotinoids and derivatives. The compounds activating or inhibiting the protein kinase pathways are less important in DNIMI-induced up-regulation in M10 cells. The apparent magnitude of time-dependent DNIMI-induced up-regulation is greater for the total cell than the cell surface nAChRs and DNIMI appears to minimize down-regulation caused by cycloheximide (to inhibit the supply of freshly synthesized receptors). Thus, DNIMI-induced up-regulation in M10 cells mainly occurs intracellularly and may involve prevention of receptor degradation.[30]

4.2. Activation of intracellular signal integration cascade

Extracellular signal-regulated kinase (ERK p44/p42), also called mitogen-activated protein kinase (MAPK), plays a critical role in signal transduction cascades from the cell surface to the nucleus. ERK is a serine/threonine protein kinase that responds to a diverse array of extracellular stimuli including neurotransmitters, hormones, growth factors, and several types of stress, and regulates many important cellular tasks such as cell growth, cell movement, differentiation, proliferation, and death.[33–35] ERK/MAPK cascades, particularly in neurons, are involved in synaptic plasticity, memory, and learning.[36,37] MAPK signaling pathways also play important roles in neuronal differentiation and commitment to survival or death, i.e., in the mechanisms underlying neurodegenerative diseases.[38–41] Neonicotinoid insecticides and their *N*-unsubstituted imine metabolites might also elicit acute intracellular response(s).

IMI, DNIMI, and nicotine induce phosphorylation of ERK (p44/p42) (activation) in a concentration-dependent manner in mouse neuroblastoma N1E-115 cells. The optimal induction is approximately twofold compared to the basal level (Fig. 2A). The concentration-dependent ERK phosphorylation elicited by the three compounds coincides with their agonist potency at the $\alpha4\beta2$ nAChR. DNIMI-induced ERK activation in N1E-115 cells is sensitive to mecamylamine (MEC) (an ion channel blocker of nAChRs) but is insensitive to α-BGT and the muscarinic antagonist atropine (Fig. 2B). The findings therefore suggest that nicotine-, DNIMI-, or IMI-induced ERK activation is mediated by primary agonist action at the $\alpha4\beta2$ nAChR in N1E-115 cells.[42]

Ca^{2+} plays an important role in regulating a great variety of cellular signaling processes. Like other higher vertebrate cells, neurons use both extracellular and intracellular sources of Ca^{2+}.[43,44] The intracellular Ca^{2+} chelator BAPTA-AM prevents the DNIMI-induced ERK phosphorylation but extracellular Ca^{2+}-free condition (EGTA) does not (Fig. 2C), suggesting that Ca^{2+} mobilization from intracellular stores is a trigger for the $\alpha4\beta2$ nAChR-initiated ERK signaling cascade in N1E-115 cells.[42]

The intracellular Ca^{2+} in neurons is stored in the endoplasmic reticulum. Although the concentration of extracellular Ca^{2+} is about 1 mM, the concentration of intracellular Ca^{2+} is usually as low as 0.1 μM and is finely regulated by various mechanisms related to physiological function. Ca^{2+} release from endoplasmic reticulum is mediated by inositol 1,4,5-trisphosphate (IP_3) and ryanodine receptors (IP_3R and RyR, respectively).[43,45] IP_3-mediated intracellular Ca^{2+} release is possibly involved in the DNIMI-induced ERK activation at least in N1E-115 cells: i.e., 2-APB, an inhibitor of IP_3-induced Ca^{2+} release, blocks the induction of phospho-ERK but ryanodine at high concentration does not. In addition, the suppression of IP_3 production by U-73122, a selective inhibitor of phospholipase C (PLC), prevents the DNIMI-activated ERK phosphorylation (Fig. 2D).[42] IP_3 and the diacylglycerol production system are associated with G-protein-coupled receptors such as the muscarinic receptor,[37,46] but DNIMI-induced ERK activation is insensitive to the muscarinic antagonist atropine. The precise mechanism(s) for the apparent interlinking between $\alpha4\beta2$ nAChR activation and IP_3-mediated Ca^{2+} release remains to be defined.

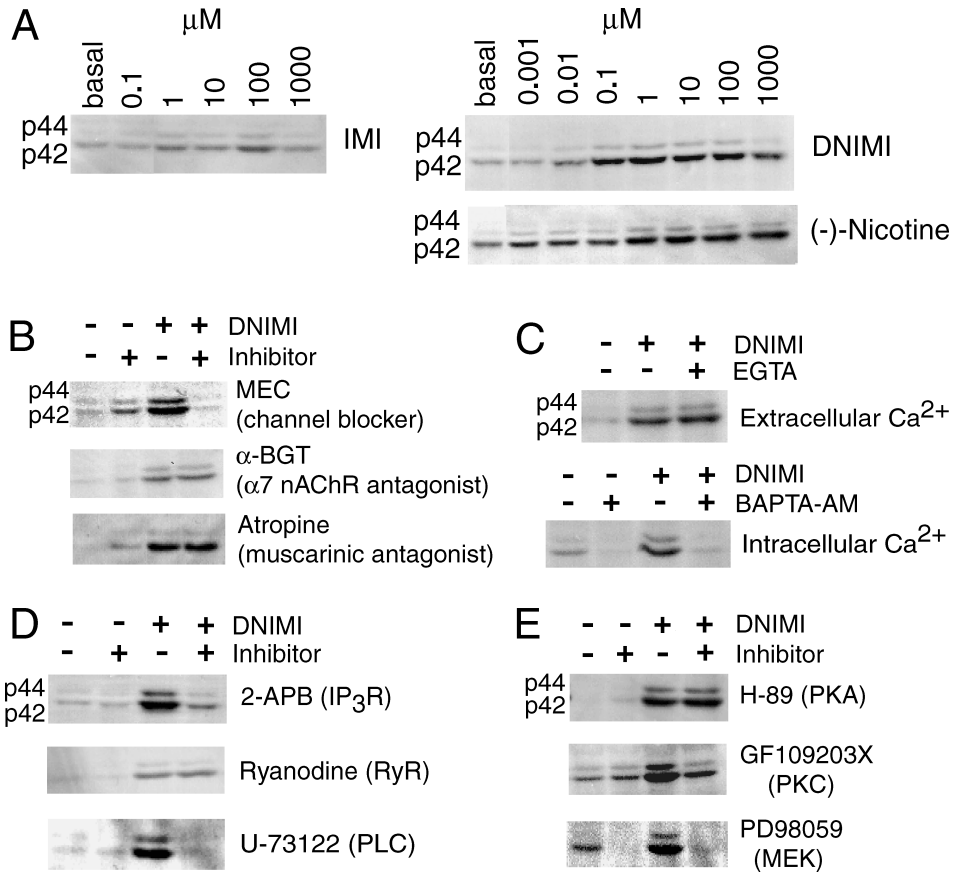

Fig. 2. Phosphorylation (activation) of extracellular signal-regulated kinase (ERK) in mouse neuroblastoma N1E-115 cells. **A**. Concentration dependence for induction of phospho-ERK (p44/p42) elicited by IMI, DNIMI, and (−)-nicotine. The induction of phospho-ERK by DNIMI or (−)-nicotine reaches an optimum at 1 to 1000 μM but the induction by IMI is significant only at 100 μM. **B**. DNIMI-induced phospho-ERK is blocked by mecamylamine (MEC) but not by α-BGT or atropine. **C**. DNIMI-induced ERK activation is prevented by the intracellular Ca^{2+} chelator BAPTA-AM but not by removal of extracellular Ca^{2+} using EGTA. **D**. Phosphorylation of ERK evoked by DNIMI exposure is blocked by 2-APB (an IP_3R inhibitor) and U-73122 (an inhibitor of PLC) but not by ryanodine (higher concentrations of ryanodine block the RyR). **E**. DNIMI-induced ERK activation is not inhibited by the PKA-selective inhibitor H-89 but is inhibited by the PKC-selective inhibitor GF109203X and the MEK (ERK kinase)-selective inhibitor PD98059. Although the data are not shown here, the total expression level of ERK (unphosphorylated form) remained unchanged in all cases. These images are from Ref. 42.

Specific inhibitors were employed to identify the intracellular signaling pathway by which DNIMI stimulates ERK activation.[42] They indicated the involvement of protein kinase C (PKC which can be stimulated by Ca^{2+}) in the DNIMI-induced ERK activation via the $\alpha4\beta2$ nAChR rather than that of protein kinase A (PKA) at least in N1E-115 cells. ERK kinase (MEK) inhibitor PD98059 completely blocks phospho-ERK induction by DNIMI-$\alpha4\beta2$ receptor interaction in N1E-115 cells (Fig. 2E), suggesting that the nicotinic signal activates ERK via a MEK-dependent cascade shared by various growth factors and mitogens. Therefore, it is proposed that the DNIMI-evoked ERK activation in N1E-115 cells involves a sequential signaling pathway with PKC, MEK kinase (possibly Raf-1), MEK, and then ERK.[42]

5. ANALGESIC AND TOXIC EFFECTS IN MICE

5.1. $\alpha4\beta2$ nAChR as the analgesic target

Nicotine has modest analgesic (antinociceptive) activity of short duration[47] while epibatidine produces profound antinociceptive effects in mice with efficacy comparable to morphine at a 200-fold lower dose.[48] The $\alpha4\beta2$ nAChR is probably the primary target for the nicotinoid-induced antinociception in the rodent preclinical pain model. The most compelling evidence is the reduced analgesic effect of nicotine in mice lacking the $\alpha4$ or $\beta2$ subunit.[49] In the action of nicotine, the pathway from the pedunculopontine tegmental nucleus to the nucleus raphe magnus is very sensitive to centrally-mediated pain perception. The antinociceptive effect of nicotine may result from stimulation of presynaptic nAChRs in the nucleus raphe magnus to release acetylcholine. The released acetylcholine then stimulates postsynaptic muscarinic recep-

Table 2. Agonist potency for $\alpha4\beta2$ nAChR in M10 cells, toxicity to mice, and antinociceptive (analgesic) activity in mice[a]

Compound	Agonist action at $\alpha4\beta2$ nAChR	Toxicity to mice	Antinociception (A_{50})[d]	
	$^{86}Rb^+$ efflux EC_{50} (μM)[b]	LD_{50} (mg/kg)[c]	Abdominal constriction (mg/kg)[e]	Hot-plate (mg/kg)[f]
IMI	117	45	>30[g]	>10[g]
Thiacloprid	85.2	28	>20[g]	>20[g]
THP-CHIMI	28.6	13	2.0	3.3
DNIMI	0.90	8	>10[g]	>10[g]
Descyano-thiacloprid	0.45	1.1	>0.5[g]	>1.0[g]
(−)-Nicotine	0.97	7	3.8	1.2
(±)-Epibatidine	0.012	0.08	0.003	0.007

[a] Data from Ref. 53. [b] Induction of specific $^{86}Rb+$ efflux from intact M10 cells that express $\alpha4\beta2$ nAChR. [c] Intraperitoneal administration. [d] Dose of test compound to produce 50% antinociception. [e] Inhibition of abdominal constriction induced by 0.6% acetic acid (intraperitoneal route). [f] Mice were placed individually on the hot-plate apparatus maintained at 52.5°C. [g] Highest doses are near LD_{50} levels or observation of toxic signs.

tors, which activate descending pathways to the dorsal horn of the spinal cord.[50–52]

5.2. Antinociceptive action of neonicotinoids and derivatives

DNIMI and descyano-thiacloprid show high agonist potency at the $\alpha4\beta2$ nAChR comparable to or greater than that of nicotine yet having no antinociceptive activity in the mice abdominal constriction and hot-plate tests (Table 2). This might result from action at a different pathway(s) rather than the antinociceptive center in mouse brain. IMI and thiacloprid are also inactive as analgesics but this could be due to low agonist potency.[53]

A nitromethylene neonicotinoid, *N*-(6-chloropyridin-3-ylmethyl)-2-nitromethylene-tetrahydro-1,3-pyrimidine (THP-CHIMI), surprisingly produces clear antinociceptive effects in both abdominal constriction and hot-plate tests and the efficacy and potency are similar to nicotine (Table 2). This analgesic activity in the mouse hot-plate test persists longer than that of nicotine or epibatidine. Interestingly this compound shows low agonist potency at the $\alpha4\beta2$ nAChR (about 30- or 2400-fold less active than nicotine or epibatidine, respectively).[53] However, THP-CHIMI is similar to nicotine in agonist potency at $\alpha3$-containing nAChRs expressed in human neuroblastoma SH-SY5Y cells.[18] It is still debatable whether analgesic neurotransmission involves activation of the peripheral $\alpha3$-containing receptor subtype expressed in sensory neurons of the trigeminal ganglion.[54] Furthermore, THP-CHIMI shows a 20-fold higher affinity than that of nicotine to human and rat $\alpha7$ nAChRs from SH-SY5Y cells and rat brain.[18] The antinociception induced by epibatidine[48] or nicotine is definitely antagonized by pretreating mice with mecamylamine. Unexpectedly, mecamylamine pretreatment does not antagonize the THP-CHIMI-induced analgesic effect (Table 3).[53] The contribution of $\alpha7$ nAChRs to nicotinic analgesia is not clear since **a**) the $\alpha7$ receptor-selective antagonist methyllycaconitine does not prevent nicotine-induced antinociception in the rat tail-flick test with the intracerebroventricular route[55] but does antagonize with spinal canal injection in mice[56] and **b**) $\alpha7$ receptor agonist-mediated behaviors are sensitive to mecamylamine.[57]

Table 3. Effect of mecamylamine on antinociception induced by (−)-nicotine and the nitromethylene neonicotinoid (THP-CHIMI) in the mouse hot-plate test[a]

Pretreatment[c]	Maximum possible effect (%)[b]	
	(−)-Nicotine 2 mg/kg	THP-CHIMI 5 mg/kg
Saline	67	64
Mecamylamine (1 mg/kg)	27[d]	70[e]

[a] Data from Ref. 53. [b] 10 min posttreatment. [c] Pretreated 5 min before the test compound administered at a different intraperitoneal site. [d] $P<0.01$. [e] Combined toxicity is a limiting factor in test with mecamylamine and THP-CHIMI, i.e., no mortality with either compound alone (mecamylamine at 0.5, 1, or 2 mg/kg and THP-CHIMI at 3 or 5 mg/kg) but 38% mortality (after 24 hr) with mecamylamine at 1 mg/kg and THP-CHIMI at 5 mg/kg and 80–100% mortality (within 10–15 min) with mecamylamine at 2 mg/kg and THP-CHIMI at 3 mg/kg.

5.3. Toxicity

The intraperitoneal toxicity of neonicotinoids and derivatives in mice is centrally mediated based on the poisoning signs

(tremors, sedation, reduced locomotion, and seizures) similar to those of nicotine and epibatidine and it correlates with their agonist action at the $\alpha4\beta2$ nAChR subtype as the primary target in brain ($r=0.93$) (Table 2).[18,53]

5.4. Relationship between analgesic and toxic effects

The neonicotinoids and their derivatives (except THP-CHIMI) do not induce antinociception, although DNIMI and descyano-thiacloprid display high agonist potency at the $\alpha4\beta2$ receptor. This result may be rationalized in two ways. First, their agonist action at the $\alpha4\beta2$ receptor as a primary target in brain leads to the centrally-mediated toxic effects rather than altering the pain sensation pathway(s). Second, the analgesic effect produced by nicotinic agonists (delivered either systemically or spinally) accompanies significant algesic (nociceptive) and cardiovascular responses.[58–61] Thus, the balance of excitatory and inhibitory effects of nicotinic agonists on pain sensation pathway(s) may help determine whether the analgesic and adverse toxic effects are mediated via the same receptor. On the other hand, a somewhat different mechanism of antinociception and a concomitant toxicity is evident for THP-CHIMI based on mecamylamine pretreatment (Table 3).[53]

6. SUMMARY

It is not clear if the toxicity of IMI in mammals is due to the parent compound or its metabolite. IMI is highly absorbed in a human intestinal cell model[62] and the entry of DNIMI into mouse brain is confirmed by an *ex vivo* study,[63] suggesting potential effects on mammals. The nAChR may also be up-regulated in mammals by chronic exposure to neonicotinoid insecticides or their metabolites. They also elicit acute intracellular response(s) in mammalian cells. Low levels of these compounds clearly activate the ERK cascade via the nAChR and intracellular Ca^{2+} mobilization. Therefore, stimulation of the nervous system in acute or sustained exposure to these chemicals may lead to synaptic plasticity or attenuate neuronal functions. The neonicotinoid insecticides and their *N*-unsubstituted imines show no analgesic activity but the nitromethylene neonicotinoid-produced antinociception is mediated in a different manner or at a target site other than the $\alpha4\beta2$ nAChR for nicotine or epibatidine. Clearly, multifaceted approaches are required to understand the fundamental aspects of the actions of neonicotinoid insecticides in mammals.

ACKNOWLEDGMENTS

The author expresses his gratitude to Professor John E. Casida of the University of California at Berkeley and emeritus Professor Izuru Yamamoto of the Tokyo University of Agriculture for their warm-hearted and everlasting support. The author thanks Professor Shinzo Kagabu of the Gifu University for valuable advice and encouragement.

REFERENCES

1) J. E. Casida and G. B. Quistad: *Annu. Rev. Entomol.* **43**, 1–16 (1998).
2) J. E. Casida and G. B. Quistad: *J. Pestic. Sci.* **29**, 81–86 (2004).
3) S. Kagabu: *Rev. Toxicol*. **1**, 75–129 (1997).
4) I. Yamamoto and J. E. Casida (eds.): "Nicotinoid Insecticides and the Nicotinic Acetylcholine Receptors," Springer, Tokyo, (1999).
5) K. Matsuda, S. D. Buckingham, D. Kleier, J. J. Rauh, M. Grauso and D. B. Sattelle: *Trends Pharmacol. Sci*. **22**, 573–580 (2001).
6) S. Kagabu: "Chemistry of Crop Protection, Progress and Prostects in Science and Regulation," ed. by G. Voss and G. Ramos, Wiley-VCH, Weinheim, pp. 193–212 (2003).
7) M. Tomizawa and J. E. Casida: *Annu. Rev. Entomol*. **48**, 339–364 (2003).
8) M. Tomizawa, D. L. Lee and J. E. Casida: *J. Agric. Food Chem*. **48**, 6016–6024 (2000).
9) M. Tomizawa, N. Zhang, K. A. Durkin, M. M. Olmstead and J. E. Casida: *Biochemistry* **42**, 7819–7827 (2003).
10) N. Zhang, M. Tomizawa and J. E. Casida: *J. Med. Chem*. **45**, 2832–2840 (2002).
11) N. Zhang, M. Tomizawa and J. E. Casida: *Bioorg. Med. Chem. Lett.* **13**, 525–527 (2003).
12) J.-P. Corringer, N. Le Novère and J.-P. Changeux: *Annu. Rev. Pharmacol. Toxicol*. **40**, 431–458 (2000).
13) D. A. Dougherty: *Science* **271**, 163–168 (1996).
14) W. Zhong, J. P. Gallivan, Y. Zhang, L. Li, H. A. Lester and D. A. Dougherty: *Proc. Natl. Acad. Sci. USA* **95**, 12088–12093 (1998).
15) D. A. Dougherty and H. A. Lester: *Nature* **411**, 252–255 (2001).
16) G. K. Lloyd and M. Williams: *J. Pharmacol. Exp. Ther*. **292**, 461–467 (2000).
17) M. N. Romanelli and F. Gualtieri: *Med. Res. Rev*. **23**, 393–426 (2003).
18) M. Tomizawa and J. E. Casida: *Br. J. Pharmacol*. **127**, 115–122 (1999).
19) M. J. Marks, J. A. Stitzel and A. C. Collins: *J. Pharmacol. Exp. Ther.* **235**, 619–628 (1985).
20) R. D. Schwartz and K. J. Kellar: *J. Neurochem*. **45**, 427–433 (1985).
21) M. E. M. Benwell, D. J. K. Balfour and J. M. Anderson: *J. Neurochem*. **50**, 1243–1247 (1988).
22) C. M. Flores, S. W. Rogers, L. A. Pabreza, B. B. Wolfe and K. J. Kellar: *Mol. Pharmacol.* **41**, 31–37 (1992).
23) J. R. Pauly, M. J. Marks, S. D. Gross and A. C. Collins: *J. Pharmacol. Exp. Ther*. **258**, 1127–1136 (1991).
24) M. J. Marks, J. R. Pauly, S. D. Gross, E. S. Deneris, I. Hermans-Borgmeyer, S. F. Heinemann and A. C. Collins: *J. Neurosci*. **12**, 2765–2784 (1992).
25) H. N. Nguyen, B. A. Rasmussen and D. C. Perry: *J. Pharmacol. Exp. Ther*. **307**, 1090–1097 (2003).
26) X. Peng, V. Gerzanich, R. Anand, P. J. Whiting and J. Lindstrom: *Mol. Pharmacol*. **46**, 523–530 (1994).
27) M. Bencherif, K. Fowler, R. J. Lukas and P. M. Lippiello: *J. Pharmacol. Exp. Ther*. **275**, 987–994 (1995).
28) M. Gopalakrishnan, E. J. Molinari and J. P. Sullivan: *Mol. Phar-*

macol. **52**, 524–534 (1997).
29) P. Whiteaker, C. G. V. Sharples and S. Wonnacott: *Mol. Pharmacol*. **53**, 950–962 (1998).
30) M. Tomizawa and J. E. Casida: *Toxicol. Appl. Pharmacol*. **169**, 114–120 (2000).
31) B. Buisson and D. Bertrand: *J. Neurosci*. **21**, 1819–1829 (2001).
32) J. R. Pauly, M. J. Marks, S. F. Robinson, J. L. van de Kamp and A. C. Collins: *J. Pharmacol. Exp. Ther*. **278**, 361–369 (1996).
33) H. J. Schaeffer and M. J. Weber: *Mol. Cell. Biol*. **19**, 2435–2444 (1999).
34) G. Pearson, F. Robinson, T. B. Gibson, B.-E. Xu, M. Karandikar, K. Berman and M. H. Cobb: *Endocr. Rev*. **22**, 153–183 (2001).
35) J. M. English and M. H. Cobb: *Trends Pharmacol. Sci*. **23**, 40–45 (2002).
36) S. S. Grewal, R. D. York and P. J. S. Stork: *Curr. Opin. Neurobiol*. **9**, 544–553 (1999).
37) J. D. Sweatt: *J. Neurochem*. **76**, 1–10 (2001).
38) S. Traverse, N. Gomez, H. Paterson, C. Marshall and P. Cohen: *Biochem. J.* **288**, 351–355 (1992).
39) Z. Xia, M. Dickens, J. Raingeaud, R. J. Davis and M. E. Greenberg: *Science* **270**, 1326–1331 (1995).
40) B. Pettmann and C. E. Henderson: *Neuron* **20**, 633–647 (1998).
41) K. T. Dineley, M. Westerman, D. Bui, K. Bell, K. H. Ashe and J. D. Sweatt: *J. Neurosci*. **21**, 4125–4133 (2001).
42) M. Tomizawa and J. E. Casida: *Toxicol. Appl. Pharmacol*. **184**, 180–186 (2002).
43) M. J. Berridge: *Neuron* **21**, 13–26 (1998).
44) V. Sorrentino and R. Rizzuto: *Trends Pharmacol. Sci*. **22**, 459–464 (2001).
45) K. Mikoshiba: *Curr. Opin. Neurobiol*. **7**, 339–345 (1997).
46) E. D. Roberson, J. D. English, J. P. Adams, J. C. Selcher, C. Kondratick and J. D. Sweatt: *J. Neurosci*. **19**, 4337–4348 (1999).
47) H. L. Tripathi, B. R. Martin and M. D. Aceto: *J. Pharmacol. Exp. Ther.* **221**, 91–96 (1982).
48) B. Badio and J. W. Daly: *Mol. Pharmacol*. **45**, 563–569 (1994).
49) L. M. Marubio, M. del M. Arroyo-Jimenez, M. Cordero-Erausquin, C. Léna, N. Le Novère, A. de K. D'Exaerde, M. Huchet, M. I. Damaj and J.-P. Changeux: *Nature* **398**, 805–810 (1999).
50) E. T. Iwamoto: *J. Pharmacol. Exp. Ther*. **251**, 412–421 (1989).
51) E. T. Iwamoto: *J. Pharmacol. Exp. Ther*. **257**, 120–133 (1991).
52) D. T. Rogers and E. T. Iwamoto: *J. Pharmacol. Exp. Ther*. **267**, 341–349 (1993).
53) M. Tomizawa, A. Cowan and J. E. Casida: *Toxicol. Appl. Pharmacol*. **177**, 77–83 (2001).
54) C. M. Flores and K. M. Hargreaves: "Neuronal Nicotinic Receptors: Pharmacology and Therapeutic Opportunities," ed. by S. P. Arneric and J. D. Brioni, Wiley-Liss, New York, pp. 359–378 (1998).
55) T. S. Rao, L. D. Correa, R. T. Reid and G. K. Lloyd: *Neuropharmacology* **35**, 393–405 (1996).
56) M. I. Damaj, M. Fei-Yin, M. Dukat, W. Glassco, R. A. Glennon and B. R. Martin: *J. Pharmacol. Exp. Ther*. **284**, 1058–1065 (1998).
57) E. M. Meyer, E. T. Tay, R. L. Papke, C. Meyers, G.-L. Huang and C. M. De Fiebre: *Brain Res*. **768**, 49–56 (1997).
58) S. R. Hamann and W. R. Martin: *J. Pharmacol. Exp. Ther*. **261**, 707–715 (1992).
59) I. M. Khan, T. L. Yaksh and P. Taylor: *Brain Res*. **753**, 269–282 (1997).
60) M. W. Decker and M. D. Meyer: *Biochem. Pharmacol*. **58**, 917–923 (1999).
61) I. M. Khan, S. Stanislaus, L. Zhang, P. Taylor and T. L. Yaksh: *J. Pharmacol. Exp. Ther*. **297**, 230–239 (2001).
62) J.-L. Brunet, M. Maresca, J. Fantini and L. P. Belzunces: *Toxicol. Appl. Pharmacol*. **194**, 1–9 (2004).
63) S.-L. Chao and J. E. Casida: *Pestic. Biochem. Physiol*. **58**, 77–88 (1997).