LOPHOTOXIN-INSENSITIVE NEMATODE NICOTINIC ACETYLCHOLINE RECEPTORS

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Summary

Nematode nicotinic acetylcholine receptors (nAChRs) are molecular targets of several anthelmintic drugs. Studies to date on *Caenorhabditis elegans* and *Ascaris suum* have demonstrated atypical pharmacology with respect to nAChR antagonists, including the finding that κ-bungarotoxin is a more effective antagonist than α-bungarotoxin on *Ascaris* muscle nAChRs. Lophotoxin and its naturally occurring analogue bipinnatin B block all vertebrate and invertebrate nAChRs so far examined. In the present study, the effects on nematode nAChRs of bipinnatin B have been examined. The *Ascaris suum* muscle cell nAChR was found to be insensitive to $30\,\mu\mathrm{mol}\,\mathrm{l}^{-1}$ bipinnatin B, a concentration that is highly effective on other nAChRs. To our knowledge, this is the first demonstration of a nAChR that is insensitive to one of the lophotoxins. *Xenopus laevis* oocytes injected with *C. elegans* polyadenylated, poly(A)+, mRNA also expressed

bipinnatin-B-insensitive levamisole responses, which were, however, blocked by the nAChR antagonist mecamylamine ($10\,\mu\mathrm{mol}\,\mathrm{l}^{-1}$). In contrast to the findings for nematode receptors, bipinnatin B ($30\,\mu\mathrm{mol}\,\mathrm{l}^{-1}$) was effective in blocking mouse muscle nAChRs expressed in *Xenopus laevis* oocytes and native insect nAChRs. A possible explanation for insensitivity of certain nematode nAChRs to lophotoxins is advanced based on the sequence of an α-like *C. elegans* nAChR subunit in which tyrosine-190 (numbering based on the *Torpedo californica* sequence), a residue known to be critical for lophotoxin binding in vertebrate nAChRs, is replaced by a proline residue.

Key words: nicotinic acetylcholine receptor, *Caenorhabditis elegans*, *Ascaris suum*, lophotoxins, bipinnatin B.

Introduction

Caenorhabditis elegans is an organism well-suited to the study of fundamental questions in neurobiology, as its nervous system consists of only 302 cells (381 cells in males) and has been mapped in its entirety (White *et al.* 1986). Furthermore, the lineage of every cell in the organism is known (Sulston and Horvitz, 1977), and sequencing of the entire genome is under way (Wilson *et al.* 1994; Hodgkin *et al.* 1995). The *C. elegans* genetic map is more complete than that of any other eukaryote, and viable mutants of nicotinic acetylcholine receptors (nAChRs) have been isolated (Lewis *et al.* 1980; Fleming *et al.* 1993). Several methods have been used to identify nAChR subunits in *C. elegans*: (a) the use of γ-irradiation-generated mutants resistant to the anthelmintic drug and cholinergic receptor ligand levamisole (Fleming *et al.* 1993); (b) screening for homology to the *Drosophila melanogaster* nAChR subunit ARD (Fleming, 1991; Fleming *et al.* 1993; Lewis and Fleming,

1992; Squire *et al.* 1995); (c) the study of neural degeneration mutants (Treinin and Chalfie, 1995); and (d) exploitation of the genome sequencing project (see Hodgkin *et al.* 1995). In addition, a putative nAChR subunit from the parasitic nematode *Onchocerca volvulus* has been partially sequenced (Ajuh and Egwang, 1994). Five nAChR subunit genes (*unc-38*, *unc-29*, *lev-1*, *acr-2* and *deg-3*) of *C. elegans* have so far been cloned and sequenced (Fleming, 1991; Fleming *et al.* 1993; Squire *et al.* 1995; Treinin and Chalfie, 1995). Investigations of recombinant nAChRs in transient expression systems such as *Xenopus laevis* oocytes have begun (Squire *et al.* 1995), and it will be of interest to compare these results with findings for *in vivo* nematode nAChRs. The small size of *C. elegans* cells, and the toughness of the nematode cuticle, make electrophysiology of *in situ* nAChRs in this model organism difficult using current technology, although initial *in*

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situ electrophysiological studies have been attempted (Lockery *et al.* 1992; Avery *et al.* 1995).

The larger nematodes of the genus *Ascaris* (*A. suum* and *A. lumbricoides*) closely resemble *C. elegans* in their neuroanatomy, but have more accessible cells, and many of the predictions about *C. elegans* neuronal function have been confirmed by experiments on *Ascaris* (Chalfie and White, 1988; Holden-Dye and Walker, 1994). Acetylcholine (ACh) is a major excitatory neurotransmitter acting on *Ascaris* muscle, and nAChRs are present, with an agonist profile as follows: DMPP (1,1-dimethyl-4-phenyl-piperazinium) > ACh > carbachol > nicotine ≫ muscarone (Rozhkova *et al.* 1980; Colquhoun *et al.* 1991; Pennington and Martin, 1990). The anthelmintic drug levamisole is a potent agonist at these receptors and at high concentrations can lead to open-channel block of activated channels (Pennington and Martin, 1990; Robertson and Martin, 1993). Other anthelmintics, morantel and pyrantel, are also extremely potent agonists at these receptors (Harrow and Gration, 1985).

There is considerable conservation in nAChRs between vertebrate species. α-Subunits, which play a central role in ACh binding (Karlin, 1993), are highly conserved, with about 80 % identity between bovine and *Torpedo californica* amino acid sequences (Claudio, 1990). In α-subunits, several highly conserved residues, Tyr-93, Trp-149, Tyr-190, Cys-192, Cys-193 and Tyr-198, are thought to contribute to the binding of ACh (Kao *et al.* 1984; see Karlin, 1993).

Of the fully sequenced *C. elegans* nAChR subunit cDNAs, *deg-3* and *unc-38* are proposed to be α-subunits as they contain the two characteristic adjacent cysteines at positions equivalent to 192 and 193 in the *T. californica* nAChR α-subunit (Treinin and Chalfie, 1995; Fleming *et al.* 1996). The amino acid sequence around the vicinal cysteines of UNC-38 differs from that observed in most other α-subunits sequenced to date in that the Tyr-190 is replaced by a proline (Pro-190) (Fleming, 1991; Fleming *et al.* 1993). The only other putative nAChR α-subunit from which Tyr-190 is absent is the vertebrate α5-subunit which, although incapable of independent expression, can co-assemble with α4 and β2 to generate a functional nAChR distinct from that formed by α4 and β2 alone (Ramirez-Latorre *et al.* 1996). A class of toxins active on vertebrate nAChRs, which includes lophotoxin and naturally occurring analogues (e.g. bipinnatin B), is known to react specifically and covalently with this tyrosine residue (Abramson *et al.* 1989). Known generically as lophotoxins, members of this family of toxins have all been isolated from various species of soft corals. In most cases, the blocking actions of the lophotoxins are not readily reversible, a finding consistent with their demonstrated interaction with Tyr-190 of vertebrate nAChRs (Abramson *et al.* 1988, 1989). Studies on the kinetics and chemistry of the lophotoxins have begun to elucidate the mechanisms by which these toxins react with Tyr-190 (Groebe *et al.* 1994; Hyde *et al.* 1995*a,b*).

We therefore decided to test one of the lophotoxins, bipinnatin B (see Fig. 1), on *in situ A. suum* muscle nAChRs.

Xenopus laevis oocytes expressing mouse muscle nAChRs and *C. elegans* nAChRs were also tested for their sensitivity to ACh, levamisole and bipinnatin B.

Materials and methods
Electrophysiology of Ascaris suum *muscle nAChRs*

Methods for electrophysiological recording of membrane potential and input resistance for *A. suum* muscle strips have been described previously (Holden-Dye *et al.* 1989). Briefly, the worms were kept alive in artificial perienteric fluid [APF, composition (in mmol l^{-1}): NaCl, 67; Tris-HCl, 5; sodium acetate, 67; KCl, 3; CaCl$_2$, 3; MgCl$_2$, 15.7; glucose, 3; pH 7.6]. Strips of body wall were isolated, pinned to the floor of a Sylgard experimental chamber, and the exposed muscle fibres impaled with two glass microelectrodes containing 4 mmol l^{-1} potassium acetate and 10 mmol l^{-1} KCl (resistance approximately 10 MΩ). Recordings were made using an Axoclamp 2A (Axon Instruments) voltage-clamp amplifier. Current pulses were injected intracellularly through one of the electrodes, thereby monitoring changes in membrane potential and input resistance.

A gravity perfusion system was used to perfuse the preparation with APF, and drugs were dissolved in APF. Bipinnatin B was stored in 100 % dimethylsulphoxide (DMSO) and diluted in APF. All recordings were performed with 1 % DMSO present, and no differences in agonist responses were observed before and after addition of DMSO to the perfusion medium.

Polyadenylated mRNA from Caenorhabditis elegans

C. elegans were grown according to the method of Sulston and Hodgkin (1988). Mixed-stage worms (1 g) were washed off the agar plates in M9 buffer (Sulston and Hodgkin, 1988) into a pre-cooled mortar on dry ice, ground in liquid nitrogen, the powder transferred to a 50 ml sterile Falcon tube and 12 ml of GTC solution [5 mol l^{-1} guanidium isothiocyanate, 50 mmol l^{-1} Tris-HCl (pH 7.4), 10 mmol l^{-1} EDTA, 2 % sarkosyl and 1 % 2-mercaptoethanol] was added. The solution was homogenized using a Polytron (type PT 10 OD) at medium to high speed for 30 s and centrifuged at 5000 revs min^{-1} (approximately 2600 g) at 4 °C for 10 min (Sorval RC-5B refrigerated centrifuge with a Sorval GS3 rotor). The supernatant was carefully layered on top of a cushion of 3 ml of sterile CsCl$_2$ (5.7 mol l^{-1}) in a Beckman ultracentrifuge tube and spun at 32 000 revs min^{-1} (approximately 130 000 g) for 18 h (Beckman L8-70, using a SW40 rotor). The supernatant was removed and the RNA pellet was resuspended in 10 mmol l^{-1} Tris-HCl, 1 mmol l^{-1} EDTA, pH 7.4 (TE) and precipitated with ethanol. The polyadenylated mRNA, poly(A)$^+$ mRNA, was isolated using an Oligo dT-cellulose column (BioRad) and standard protocols (Sambrook *et al.* 1989).

cRNA encoding mouse muscle nAChR subunits

cDNA clones encoding the four mouse muscle (α$_1$, β$_1$, γ$_1$, δ$_1$) nAChR subunits were kindly provided by Professor S.

Heinemann, Salk Institute, San Diego, USA. The clones were transcribed *in vitro* using a Riboprobe kit obtained from Promega (catalogue no. P1221).

Preparation of the Xenopus laevis *oocytes*

Oocytes were surgically removed from the frog and kept in SOS medium (in $mmol\,l^{-1}$: NaCl, 100; Hepes, 5; $CaCl_2$, 1.8; $MgCl_2$, 1; KCl, 2; pH 7.6). After defolliculation, they were injected with 46 nl of RNA [encoding mouse nAChR subunits or *C. elegans* total poly(A)$^+$ mRNA] using a manual injector with a 10 µl microdispenser glass needle (Drummond). The concentration of the injected RNA solution was $1\,mg\,ml^{-1}$. The oocytes were assayed for functional expression 2–7 days later using electrophysiology.

Electrophysiology of oocytes injected with Caenorhabditis elegans *total poly(A)$^+$ mRNA and mouse muscle nAChR cDNA-derived RNA*

For electrophysiology, the oocytes were impaled with glass (GC150 GF-10, Clark Electromedical) microelectrodes filled with $1\,mol\,l^{-1}$ KCl (3–5 MΩ) and voltage-clamped using a Geneclamp 500 amplifier (Axon Instruments). Membrane currents recorded from oocytes expressing mouse muscle nAChRs were monitored on an oscilloscope (Nicolet 3091) and recorded on a chart recorder (Gould BS 272). Drugs and medium were applied *via* a perfusion pump (Pharmacia LKB pump P-1). All recordings were obtained in the presence of 1 % DMSO, which by itself had no effect.

Chemicals

DMSO, acetylcholine, nicotine, mecamylamine and *d*-tubocurarine were obtained from Sigma Chemical Company Ltd. Bipinnatin B (see Fig. 1) was purified, characterized and stored as previously described (Hyde *et al.* 1995*a*).

Results

Bipinnatin B blocks expressed mouse muscle nAChRs and native Periplaneta americana *nAChRs*

Xenopus laevis oocytes expressing mouse muscle nAChRs formed by co-injection of cRNA encoding α_1-, β_1-, γ_1- and δ_1-subunits responded to applications of ACh with large inward currents. The amplitude of the response to ACh was reduced reversibly in the presence of *d*-tubocurarine ($10\,µmol\,l^{-1}$ for 15 min). A 1 h application of bipinnatin B ($30\,µmol\,l^{-1}$) completely abolished the response to ACh (Fig. 1) and this did not recover even after extensive washing (30–60 min) (not shown) (*N*=3).

Bipinnatin B has been shown to block the response to nicotine in the fast coxal depressor motor neurone (D_f) of the cockroach *Periplaneta americana* (Bai *et al.* 1993). The response to nicotine ($10\,µmol\,l^{-1}$) was reduced to about 20 % of the amplitude of control responses after a 26 min application of $30\,µmol\,l^{-1}$ bipinnatin B (Bai *et al.* 1993). For this study, bipinnatin B was tested on D_f and, after a 15 min application of $30\,µmol\,l^{-1}$ toxin, only about 30 % of the amplitude of the
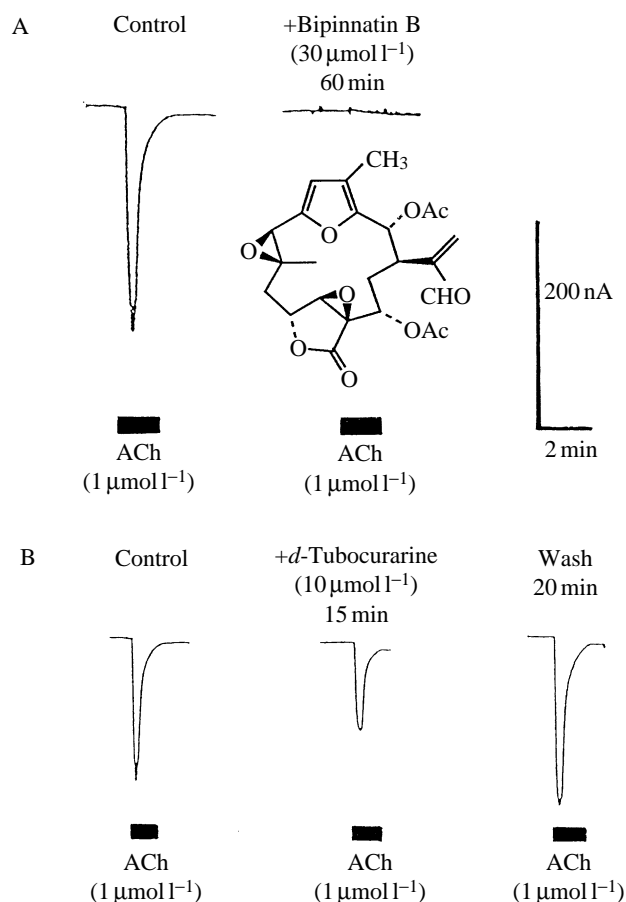

Fig. 1. Response to $1\,µmol\,l^{-1}$ acetylcholine (ACh) recorded from *Xenopus laevis* oocytes injected with mouse muscle nicotinic acetylcholine receptor (nAChR) cRNA $\alpha_1,\beta_1,\gamma_1,\delta_1$. The recordings were made under two-electrode voltage-clamp, in the presence of 1 % DMSO, with the cell clamped at −100 mV. (A) The ACh-induced current was completely blocked by a 1 h incubation with $30\,µmol\,l^{-1}$ bipinnatin B (*N*=3). Inset shows the structure of bipinnatin B. (B) The ACh-induced current was reversibly reduced in amplitude by a 15 min application of $10\,nmol\,l^{-1}$ *d*-tubocurarine (*N*=5).

control response to $10\,µmol\,l^{-1}$ nicotine was observed (Fig. 2) (*N*=3).

Ascaris suum *muscle nAChRs: block by mecamylamine and insensitivity to bipinnatin B*

A. suum muscle bag cells were depolarized in response to ACh applications. A concentration ($5\,µmol\,l^{-1}$ ACh) close to the threshold of ACh sensitivity of *Ascaris* muscle bag cells was used, as too large a response can cause the muscle strip to contract, thereby dislodging the electrodes or damaging the cell. The low concentration of ACh employed also makes this a very sensitive assay for toxin activity. *A. suum* muscle nAChRs have been found to be sensitive to a range of nicotinic antagonists such as mecamylamine, neosurugatoxin and κ-bungarotoxin, an antagonist at many vertebrate neuronal nAChRs, but are only moderately sensitive to the vertebrate muscle nAChR antagonist α-bungarotoxin (Colquhoun *et al.*
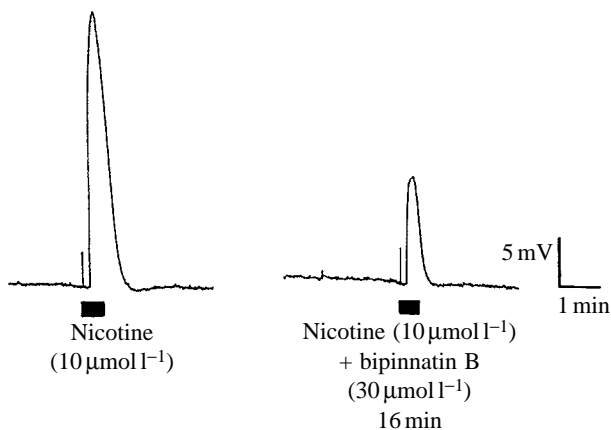
Fig. 2. Voltage recording from the cockroach *Periplaneta americana* D_f motor neurone in the metathoracic ganglion. The response to nicotine is blocked by $30\,\mu\mathrm{mol}\,l^{-1}$ bipinnatin B.

1993). The estimated IC_{50} for the blocking action of mecamylamine on *Ascaris suum* muscle nAChRs was $0.33\,\mu\mathrm{mol}\,l^{-1}$. In four preparations tested, applications of $30\,\mu\mathrm{mol}\,l^{-1}$ bipinnatin B for 20–60 min were without effect on the ACh-induced response, whether measured by conductance or by membrane potential changes (Fig. 3A). The ACh-induced responses were blocked by a 10 min application of mecamylamine ($10\,\mu\mathrm{mol}\,l^{-1}$), as illustrated in Fig. 3B.

Caenorhabditis elegans *nAChRs expressed in* Xenopus laevis *oocytes: block by mecamylamine and insensitivity to bipinnatin B*

Levamisole depolarized oocytes injected with poly(A)$^+$ mRNA from mixed stages of *C. elegans*. Very few of these oocyte injections resulted in expression, although the cells generally survived the injection well. Nevertheless, when expression did occur, inward currents were readily detected in
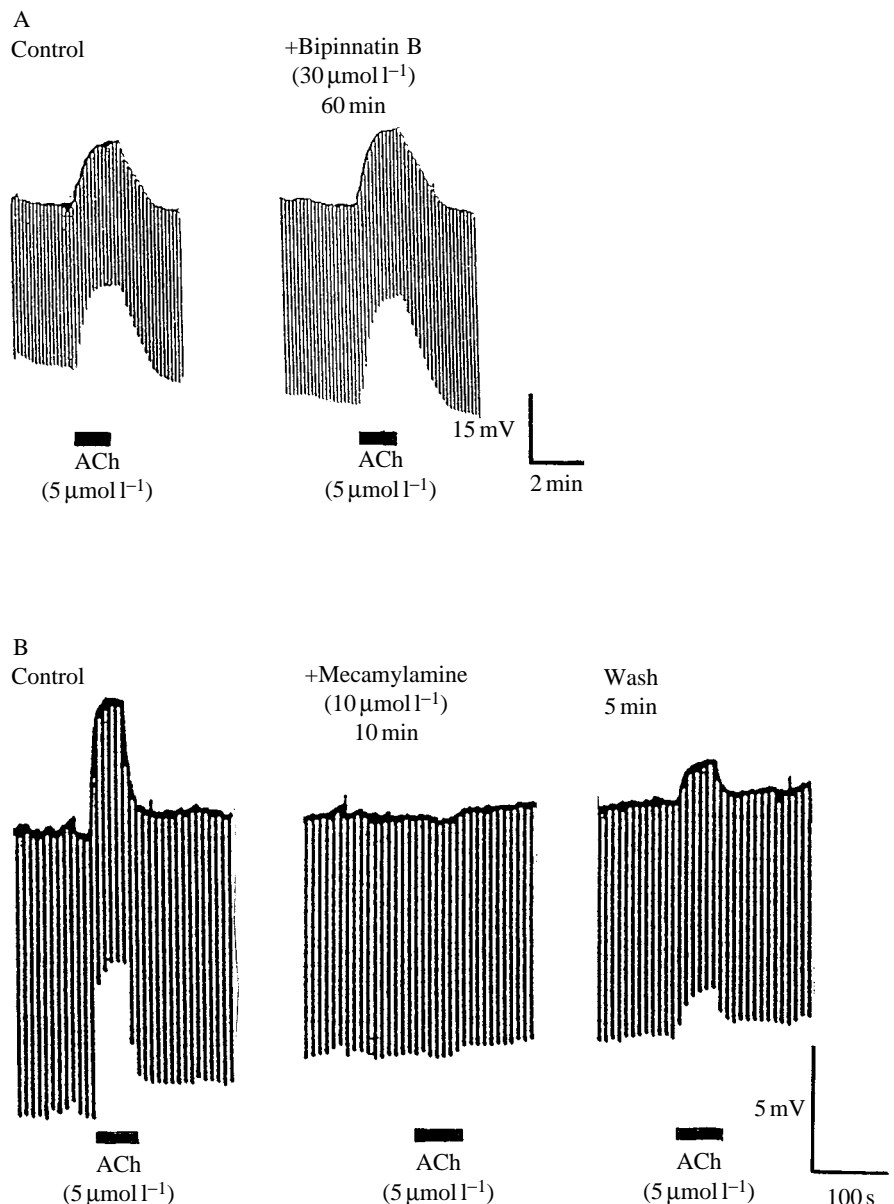

Fig. 3. (A) Two-electrode voltage recordings from *Ascaris suum* muscle cells showing changes in membrane potential and input conductance in response to bath application of $5\,\mu\mathrm{mol}\,l^{-1}$ acetylcholine (ACh). The membrane potential was approximately $-30\,\mathrm{mV}$. The downward deflections result from the current injections used to measure the input conductance change during the response to ACh. The response to ACh of *Ascaris suum* muscle nAChRs was unaffected by a 1 h application of $30\,\mu\mathrm{mol}\,l^{-1}$ bipinnatin B ($N=3$). (B) $5\,\mu\mathrm{mol}\,l^{-1}$ ACh was applied for 1 min with 1 % DMSO present in the medium and in the ligand solution. The response to ACh was blocked by prior application of $10\,\mu\mathrm{mol}\,l^{-1}$ mecamylamine ($N=3$). The block was partly reversible.

A
Control

+Bipinnatin B
($30\,\mu mol\,l^{-1}$)
20 min

+Bipinnatin B
($30\,\mu mol\,l^{-1}$)
60 min

10 nA

Levamisole
($160\,\mu mol\,l^{-1}$)

Levamisole
($160\,\mu mol\,l^{-1}$)

Levamisole
($160\,\mu mol\,l^{-1}$)

1 min

B
Control

+Mecamylamine
($10\,\mu mol\,l^{-1}$)
10 min

Wash
15 min

10 nA

Levamisole
($100\,\mu mol\,l^{-1}$)

Levamisole
($100\,\mu mol\,l^{-1}$)

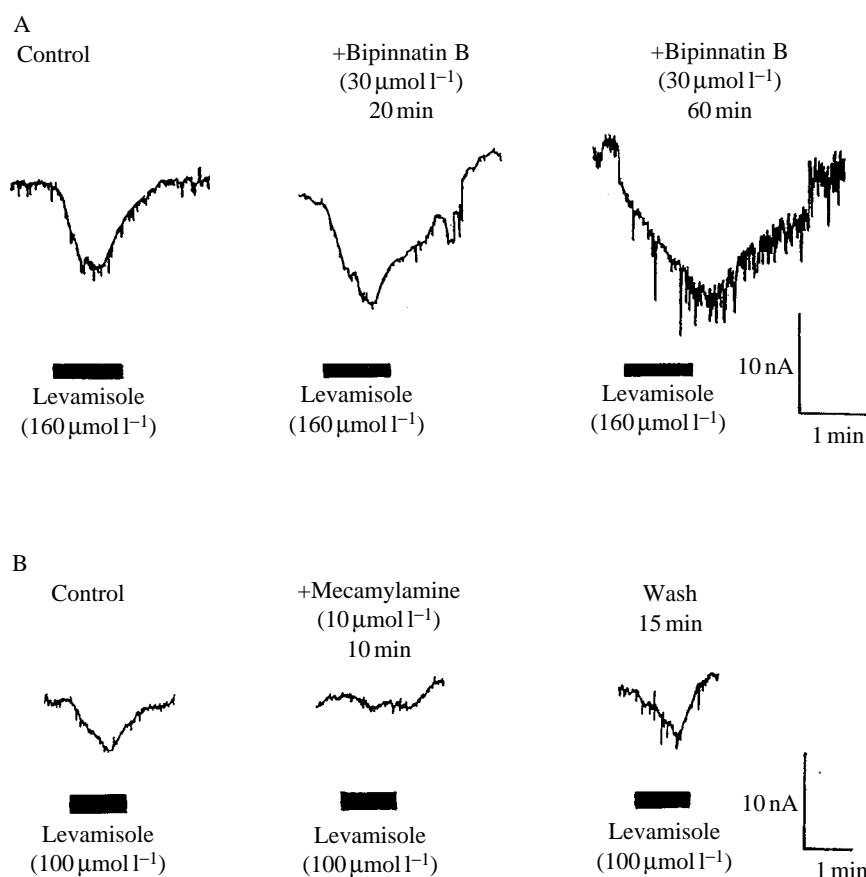Levamisole
($100\,\mu mol\,l^{-1}$)

1 min

Fig. 4. Recordings from a *Xenopus laevis* oocyte injected with *Caenorhabditis elegans* total polyadenylated mRNA. Under standard two-electrode voltage-clamp, the oocytes responded to levamisole with inward currents. (A) After a 20 min incubation with $30\,\mu mol\,l^{-1}$ bipinnatin B, the response to $160\,\mu mol\,l^{-1}$ levamisole was undiminished. Incubation for 1 h with $30\,\mu mol\,l^{-1}$ bipinnatin B still failed to block the levamisole-induced response (*N*=3). (B) The response to $100\,\mu mol\,l^{-1}$ levamisole was completely abolished by a 10 min application of $100\,\mu mol\,l^{-1}$ mecamylamine, but recovered after a 15 min wash with saline.

response to levamisole ($10–160\,\mu mol\,l^{-1}$) in two-electrode voltage-clamp experiments. The levamisole- and mecamylamine-sensitive receptors observed in oocytes injected with *C. elegans* poly(A)$^+$ mRNA appeared to be insensitive to bipinnatin B. The response to levamisole was tested after 20 min and 60 min incubations with $30\,\mu mol\,l^{-1}$ bipinnatin B and was found to be undiminished compared with the control response recorded prior to application of the toxin (Fig. 4A). Owing to the erratic expression of these receptors, combined with the limited availability of the toxin, only three cells were subjected to bipinnatin B treatment, but these results on *C. elegans* expressed nAChRs confirm the hypothesis that a lophotoxin-insensitive receptor exists in the nematodes. The levamisole-induced currents (in response to $100\,\mu mol\,l^{-1}$ levamisole) were completely blocked by a 10 min incubation with mecamylamine ($100\,\mu mol\,l^{-1}$, *N*=3). Responses to levamisole recovered within 15 min of washing with saline (Fig. 4B).

Discussion

Lophotoxin and its analogues have been found to block all nAChRs tested prior to this study (Abramson *et al.* 1991). The potent, naturally occurring analogue bipinnatin B has been shown to block vertebrate muscle and neuronal nAChRs (Luetje *et al.* 1990; Culver and Jacobs, 1984; Abramson *et al.* 1991), and both lophotoxin (Blagburn and Sattelle, 1988) and

bipinnatin B (Bai *et al.* 1993; Tornøe *et al.* 1994) blocked α-bungarotoxin-sensitive neuronal nAChRs in the cockroach nervous system. The interaction of lophotoxins with vertebrate nAChRs involves a covalent reaction with the tyrosine residue in position 190 of the α-subunit (*T. californica* numbering) (Abramson *et al.* 1989).

With the exception of UNC-38 (Fig. 5), this Tyr-190 residue is conserved in the majority of functional α-subunits cloned to date from a wide range of animal species: mouse (Wada *et al.* 1988), *Drosophila melanogaster* ALS (Bossy *et al.* 1988), *Drosophila melanogaster* SAD (Sawruk *et al.* 1990), calf, human, cobra, frog (McLane *et al.* 1991*a*), chick α_{1-4} (Bertrand *et al.* 1990), rat α_{1-4} (Boulter *et al.* 1990), locust αL1 (Marshall *et al.* 1990), *C. elegans* DEG-3 (Treinin and Chalfie, 1995) and the avian and rat α_7-subunits (Schoepfer *et al.* 1990; McLane *et al.* 1991*b*; Couturier *et al.* 1990*a*; Seguela *et al.* 1993).

Rat and chick α_5-subunits, identified by their α-bungarotoxin binding capacities and cloned by homology screening, lack Tyr-190. Until recently, neither of these vertebrate α-subunits had been shown to form functional receptors when injected either alone or in combination with non-α-subunits (Couturier *et al.* 1990*b*; Boulter *et al.* 1990). The recent demonstration that α_5 can co-express to form a functional receptor with α_4- and β_2-subunits provides the first evidence of a functional role for α_5 (Ramirez-Latorre *et al.* 1996). The lophotoxins have not to date been tested on a nAChR recombinant receptor containing α_5.

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KKYDCCAE-IYPD    Rat α2
KKYDCCTE-IYPD    Chick α2
VFYSCCPTTPYLD    Mouse α1
VYYTCCPDTPYLD    T. californica α1
KFYSCCEEPY-LD    D. melanogaster ALS
KYYPCCAE-PYPD    D. melanogaster SAD
KYYPCCAEPY-PD    S. gregaria αL1
NYPSCCPQSAYUD    C. elegans UNC-38
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Fig. 5. Sequences around the vicinal cysteines (positions 192 and 193, *Torpedo californica* numbering) of several nAChR α-subunits. Note the absence of a tyrosine residue (Y) in position 190 in the *Canorhabditis elegans* UNC-38 sequence. The Swissprot database accession numbers of the sequences shown are as follows: rat α2 (P12389); chick α2 (PO9480); mouse α1 (PO4756) *Torpedo californica* α1 (PO2710); *Drosophila melanogaster* ALS (PO9478); *Drosophila melanogaster* SAD (P17644); *Schistocerca gregaria* αL1 (P23414). The sequence for *Caenorhabditis elegans* UNC-38 is taken from EMBL: X98600.

UNC-38 is the only other functional nAChR α-subunit which lacks the Tyr-190 (EMBL: X98600; see also Squire *et al.* 1995). This change from a tyrosine to a proline (Fig. 5) could conceivably explain the observed insensitivity to bipinnatin B. As there is a tyrosine in position 189 (*T. californica* numbering) in the UNC-38 sequence, it is possible that this residue is 'phase-shifted' by a proline insert. However, Tyr-189 of the *Torpedo californica* receptor is not covalently labelled by lophotoxins, and the presence of a tyrosine at position 189 in UNC-38 may not be sufficient for lophotoxin binding. Since proline residues can introduce bends in the secondary structure of polypeptides (Chou and Fasman, 1978), this could generate a structural change that affects ligands binding close to the ACh binding site. Both *A. suum* muscle *in situ* nAChRs and oocytes expressing whole *C. elegans* poly(A)$^+$ mRNA appear to be insensitive to bipinnatin B.

As the response in oocytes expressing whole *C. elegans* poly(A)$^+$ mRNA appears to be insensitive to the lophotoxin analogue bipinnatin B, this may imply that the oocytes have primarily expressed those nAChRs containing the UNC-38 subunit (or perhaps similar subunits lacking the Tyr-190). It could also be the case that the UNC-38-like nAChR subunits in *C. elegans* are abundant in this animal and, because of this, their effects are readily observed. Recent PCR (polymerase chain reaction) studies have shown that UNC-38 is abundant in *C. elegans* (J. Lewis, personal communication). Whether the *A. suum* muscle nAChR α-subunit possesses a tyrosine residue at position 190 remains undetermined, but the data presented here provide the first demonstration of nAChRs insensitive to the lophotoxins, and the observation that an α-subunit without Tyr-190 does exist in nematodes offers one plausible hypothesis for this novel nAChR pharmacology. This hypothesis should be readily testable using site-directed mutagenesis and expression of recombinant receptors carrying wild-type and mutated UNC-38.

If this is not the case, other amino acid sequences unique to nematode nAChR subunits may be involved, and this would also be of considerable interest in understanding toxin–receptor interactions. The newly cloned nAChR subunit of *O. volvulus* shows approximately 70% identity with *C. elegans* UNC-38. As the *O. volvulus* sequence does not contain the adjacent cysteine residues (equivalent to *T. californica* α-subunit residues 192 and 193), it is considered to be a non-α-subunit (Ajuh and Egwang, 1994). There are, however, several features included in the *O. volvulus* nAChR subunit sequence which resemble other nAChR α-subunits and are not normally present in non-α-subunits, such as the Tyr-190 residue. Given the remarkable divergence from other vertebrate and invertebrate nAChR subunits among nematode sequences, several changes could be responsible for the lack of interaction with bipinnatin B. Further studies on ligand–nAChR interactions in nematodes will be of considerable interest in the light of their unusual pharmacology and certain atypical amino acid sequences in the vicinity of the neurotransmitter binding site.

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