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Differential block of nicotinic synapses on B versus C neurones in sympathetic ganglia of frog by α-conotoxins MII and ImI

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1 The effects of two new acetylcholine receptor antagonists, α-conotoxin MII and α-conotoxin ImI, on nicotinic synaptic transmission in the 10th paravertebral sympathetic ganglion of the leopard frog (*Rana pipiens*) were examined. The preganglionic nerve was electrically stimulated (at low frequency, $\leqslant 1$ min^{-1}, to avoid use-dependent changes) while compound action potentials of B and C neurones were monitored from the postganglionic nerve.

2 α-Conotoxins MII and ImI, at low micromolar concentrations, reversibly blocked both B and C waves. α-Conotoxin MII blocked the C wave more effectively than the B wave, whereas the potency of α-conotoxin ImI was opposite that of MII. The observation that nicotinic antagonists can differentially block synaptic transmission of B versus C neurones with opposite selectivities strongly suggests that these neurones possess distinct nicotinic receptors.

3 In addition to fast and slow B waves described by others, C waves with two temporally distinguishable components were present in our recordings. Each α-conotoxin affected fast and slow B waves similarly. Likewise, toxins did not discriminate between the two components of C waves. This suggests that all neurones within each major class (B or C) may have the same nicotinic receptors.

4 Synthetic forms of α-conotoxins MII and ImI were used in the present study. Their ease of synthesis and their specificities should make these toxins useful probes to investigate the various subtypes of neuronal nicotinic acetylcholine receptors.

Keywords: α-Conotoxin; sympathetic neurone; nicotinic receptor; cholinergic synapse

Introduction

The nicotinic acetylcholine receptors (AChRs) of skeletal muscle and electroplax are among the best characterized, both in structure and in function, of all ligand-gated ion channels. In contrast, the nicotonic AChRs of neurones are less well understood; particularly with regard to their subunit composition *in situ* (Sargent, 1993).

The α-conotoxins belong to a family of structurally related peptides found in the venom of marine snails of the genus *Conus*. These peptides target nicotinic AChRs and are promising ligands for investigating different subtypes of nicotinic AChRs. Two of these toxins, α-conotoxin MII (from *Conus magus*) and α-conotoxin ImI (from *C. imperialis*), were recently described, and they discriminate between different combinations of nicotinic AChR subunits expressed in *Xenopus* oocytes injected with mRNA from cDNAs cloned from rat brain (Johnson *et al*., 1995; Cartier *et al*., 1996). The structures of these peptides are presented in Table 1.

In view of the specificities of these two α-conotoxins, we wanted to determine whether they could be used to discriminate between nicotinic AChRs in an intact physiological system. Thus, we examined the effects of α-conotoxins on nicotinic synaptic transmission in frog sympathetic ganglia. This system is particularly attractive because the ninth and tenth paravertebral sympathetic ganglia of amphibia contain two populations of principal cells, B neurones and C neurones (Nishi *et al*., 1965; Dodd & Horn, 1983a; Feldman, 1988). B neurones are larger than C neurones, and B neurones have faster conducting pre- and postganglionic axons. B neurones are innervated by axons in the connective above the 7th ganglion, whereas C neurones are innervated by axons in the rami of 7th and 8th ganglia (see review by Skok, 1973; also Dodd & Horn, 1983a). B and C neurones express both nicotinic and muscarinic ACh receptors. Nicotinic receptors mediate the fast excitatory postsynaptic potential (e.p.s.p.) in both classes of neurones. However, the muscarinic receptors in B neurones mediate a slow e.p.s.p., and those in C neurones mediate a slow inhibitory postsynaptic potential (i.p.s.p.) (Dodd & Horn, 1983b). Finally, fast synaptic transmission in the two classes of neurones in bullfrog is mediated by nicotinic AChRs with different channel kinetics (Marshall, 1986; Shen & Horn, 1995; Thigpen, 1995).

In this study, we demonstrate that fast synaptic transmission mediated by B and C neurones in sympathetic ganglia of the leopard frog are differentially blocked by α-conotoxins ImI and MII. These results strongly suggest that sympathetic B and C neurones have pharmacologically distinct forms of nicotinic acetylcholine receptors and indicate that the α-conotoxins may be useful ligands for discriminating between different nicotinic acetylcholine receptor subtypes in other neuronal systems.

Methods

Lumbar paravertebral ganglia 7 through 10 and the adjoining 10th spinal nerve were isolated from adult frogs (*Rana pipiens*) of either sex. The recording chamber was fabricated from Sylgard, and it consisted of a trough about 20 mm long, 3 mm wide and 2 mm deep which could be divided into four compartments (A–D) by three partitions (1–3) (see Figure 1). Three vertical, transverse slits were cut into the wall and floor of the trough with a 10 mm-wide razor blade. The trough was partitioned by inserting into each slot an approximately 8 mm wide × 3 mm high × 0.1 mm thick sheet of Mylar, with a V-shaped notch in the top edge. The bottom edge of each sheet penetrated the floor of the trough so the vertex of the notch rested slightly above the floor. The chain of ganglia and the 10th spinal nerve were draped over the notched partitions and pinned to the floor of the trough. A notched Mylar sheet si-

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Table 1 Amino acid sequences and disulphide bridges of α-conotoxins MI, MII and ImI

MI	Gly Arg Cys Cys His Pro Ala Cys Gly Lys Asn Tyr Ser Cys*
MII	Gly Cys Cys Ser Asn Pro Val Cys His Leu Glu His Ser Asn Leu Cys*
ImI	Gly Cys Cys Ser Asp Pro Arg Cys Ala Trp Arg Cys*

*Indicates that the α-carboxyl group is amidated. Intramolecular disulphide bonds are indicated by lines connecting cystein (Cys) residues. The disulphide bonding pattern is the same for the three peptides and is characteristic of two-loop α-conotoxins. Disulphide bridges confer on the peptides relatively compact and rigid structures. α-Conotoxins MI (McIntosh *et al*., 1982) and MII (Cartier *et al*., 1996) are found in the fish-eating *C. magus*, and α-conotoxin ImI (McIntosh *et al*., 1994) is found in the worm-eating *C. imperialis*.

milar to those dividing the trough, but inverted, was slipped into each slot beside the sheet already present. The notches of each of the two Mylar sheets in a given slot were aligned so that the juxtaposed sheets formed a partition or barrier, with a diamond-shaped aperture whose size could be varied simply by sliding one sheet past the other. The connective between the 8th and 9th ganglia passed through and occluded the aperture of the first barrier; the aperture of the second barrier was occupied by the 10th spinal nerve caudal to the ramus to the 10th ganglion; and a segment of the 10th spinal nerve near its caudal stump occupied the aperture of the third barrier. Thus in all, this created four ~30 μl compartments, each sufficiently isolated from the next to allow: (1) the fluid in each compartment to be independently maintained, and (2) electrical stimulation or recording across a given Mylar partition. As sketched in Figure 1, compartment A contained the 7th and 8th ganglia; compartment B, the 9th and 10th ganglia and the rostral stump of the 10th spinal nerve; compartment C, the middle segment of the 10th spinal nerve; and compartment D, the caudal stump of the 10th spinal nerve.

Platinum wire electrodes were placed on either side of partition 1 and served to convey supramaximal stimuli to preganglionic B and C fibres leading into the 10th (as well as 9th) ganglion. Postganglionic compound action potentials of B and C neurones in the 10th ganglion (B and C waves, respectively) were recorded from the 10th spinal nerve with Pt wire electrodes placed on either side of partition 3 and connected to a high input impedance differential A/C preamplifier (either P-15, Grass Instruments or DAM-50, WP Instruments, with 1 Hz low, and 1 kHz high, frequency filters). The recording electrode in compartment D led to the positive input of the preamplifier, while that in C led to the negative input. A Pt wire ground electrode was located in compartment B. Stimuli (1 ms rectangular voltage pulses) were provided by a stimulator (S-88, Grass Instruments) through a stimulus isolation unit. Signals were captured with a Macintosh computer (either an SE/30 or LCIII) fitted with an A/D converter (either Lab-LC, National Instruments or MacADIOS adio, GW Instruments). Stimuli were triggered and responses captured (sampling frequency 5 or 10 kHz), displayed, analysed and stored, with homemade virtual instruments constructed with the graphical programming language LabVIEW (National Instruments).

The preparation was bathed in frog Ringer solution consisting of (in mM): NaCl 111, KCl 2, $CaCl_2$ 1.8, NaHEPES 10, pH 7.4. All compartments were static baths except B which contained the 10th (and 9th) ganglion and which could be perfused at a rate of ~0.5 ml min^{-1}. To expose the 10th ganglion to drug, the perfusion in compartment B was halted, and the Ringer within it replaced with that containing drug. Solutions in all static compartments, including B during drug exposure, were manually refreshed at least every 20 min to avoid adverse effects of evaporation.

Synthetic α-conotoxins (McIntosh *et al*., 1994; Cartier *et al*., 1996) were used. (+)-tubocurarine was from Sigma Chemical Co., and dihydro-β-erythroidine was a gift of Merck & Co. All drugs were dissolved in Ringer solution. It should be noted that the recording chamber used in these experiments required as little as 30 μl of test solution and allowed drugs and toxins to be used frugally.

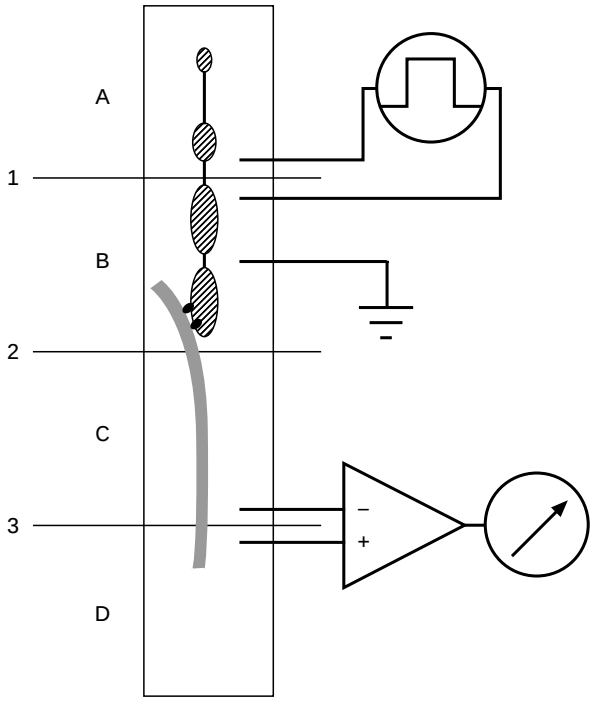

Figure 1 Sketch of the recording chamber. Ganglia are represented by hatched ovals and 10th spinal nerve by the broad stippled line. The sympathetic chain was pinned to the bottom of a Sylgard trough (~3 mm wide × 20 mm long × 2 mm deep) which was partitioned into four compartments (A, B, C and D) by three partitions (1, 2 and 3) constructed of Mylar sheets as described in Methods. The 7th and 8th ganglia were located in compartment A, and the 9th and 10th ganglia in B. The connective between the 8th and 9th ganglia passed through the aperture in partition 1. Segments of the 10th nerve (stippled line) containing postganglionic axons of neurones in the 10th ganglion traversed partitions 2 and 3. Pt wire electrodes on either side of partition 1 were used to stimulate the preganglionic nerve of the 10th ganglion, while Pt wire electrodes on either side of partition 3 were used to record compound action potentials extracellularly from the postganglionic axons in the 10th nerve. The ground electrode in B stabilized the recording and minimized stimulus artifacts. Partition 2 effectively reduced the volume of the compartment containing the 10th ganglion and thereby reduced the amount of toxin required; moreover, it helped to minimize stimulus artifacts. Only compartment B (~30 μl volume) was exposed to drugs.

Two features were used to discriminate between responses of B neurones (B waves) and C neurones (C waves); in contrast to C waves, B waves could be elicited with lower stimulus strengths and had shorter latencies (cf., Dodd & Horn, 1983a; Feldman, 1988). Since B and C waves were readily distinguishable in our recordings by their latencies alone, supramaximal stimuli sufficient to elicit both responses were used in the experiments described here. To minimize possible effects of use-dependent changes in synaptic efficacy, the preganglionic nerve was stimulated at a low frequency of $\leqslant$1 min^{-1}. All experiments were performed at room temperature (20–24°C).

Results

As previously shown by Feldman (1988), the 10th ganglion of *R. pipiens*, like that of *R. catesbiana* (Dodd & Horn, 1983a), has two types of B waves; a fast one followed immediately by a slower, smaller one. In addition to these, our recording conditions revealed C waves with two temporally distinguishable components with approximately equal amplitudes and which required similar high stimulus strengths in order to be elicited. The fast and slow B waves as well as the two-component C wave (examples of which can be seen in Figure 3) were verified to be synaptically mediated since they all were abolished when the 10th ganglion was bathed in Ringer containing low [Ca^{2+}] and high [Mg^{2+}] (not illustrated); furthermore, all were sensitive to nicotinic antagonists as discussed below.

We were specifically interested in the effects that the two new α-conotoxins, ImI and MII, had on nicotinic synaptic transmission through the ganglion. For comparison, we also examined the effects of more conventional nicotinic AChR blockers, namely (+)-tubocurarine and dihydro-β-erythroidine as well as α-conotoxin MI from *C. magus*, which has been known for some time to block neuromuscular nicotinic AChRs (Olivera *et al*., 1985). In all, seventeen 10th ganglia were examined for effects of α-conotoxin MII; subsets of these ganglia were also tested with the other antagonists as indicated below, and the results yielded a consistent picture which is summarized by the experiment illustrated in Figure 2. This figure plots, as a function of time, the peak amplitudes of fast B waves and C waves when a ganglion was sequentially exposed to five different nicotinic antagonists, with washes between exposures. The antagonists were α-conotoxin ImI (5 μM), α-conotoxin MI (5 μM), (+)-tubocurarine (10 μM) α-conotoxin MII (5 μM) and dihydro-β-erythroidine (5 μM), respectively. Representative responses before, during, and after, exposure to each antagonist are shown in Figure 3.

C waves were more readily blocked by MII than were B waves. For example, in tests involving single trials in five ganglia, 1 μM α-conotoxin MII blocked the fast C wave by 90±10%, while the fast B wave was only blocked by 59±15% (mean±s.d.). With 5 μM α-conotoxin MII, the fast C wave was blocked by 97±5%, while the fast B wave was blocked by 68±23% (single trials in 6 ganglia). In one ganglion 50 μM α-conotoxin MII was tested, and both B and C waves were blocked by 100%. In all preparations both fast and slow B waves were blocked equally well by α-conotoxin MII; likewise, both components of C waves were equally sensitive to α-conotoxin MII (see Figure 2d).

In single trials with three ganglia, 5 μM α-conotoxin ImI blocked the fast B wave by 82±5% and the fast C wave by only 14±12%. Figure 3a shows that although the effect of α-conotoxin ImI on the amplitude of the C wave was slight, it noticeably increased the latency of the response. The latency of the residual B wave was also delayed. The figure also shows that the sensitivities of both fast and slow B waves to α-conotoxin ImI were very similar, as were the sensitivities of both components of the C wave.

The effects of other nicotinic antagonists on the sympathetic responses were determined in order to compare their effects with those of α-conotoxin MII and ImI. Dihydro-β-erythroidine blocked both B and C waves about equally well; in one ganglion 0.5 μM dihydro-β-erythroidine blocked B waves by 58% and C waves by 46%, and in another ganglion 5 μM blocked both B and C waves by >90% (see Figure 3e). In two other ganglia 10 μM dihydro-β-erythroidine blocked both B and C waves by 100%. However, invariably when dihydro-β-erythroidine was washed out, B waves recovered more quickly than did C waves (see Figure 2). (+)-Tubocurarine 10 μM blocked the fast B wave by 98±4% and the C wave by 24±3% (single trials in 2 ganglia). In this respect, the specificity of (+)-tubocurarine resembles that of α-conotoxin ImI

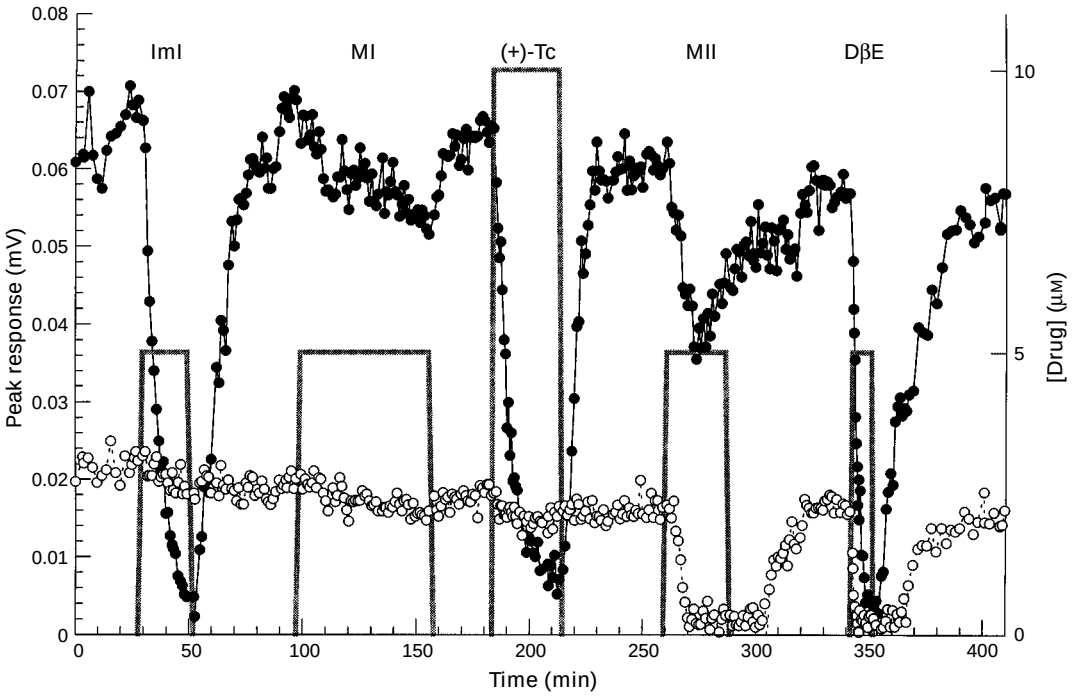

Figure 2 Nicotinic antagonists can differentially block synaptic transmission of B and C neurones in the 10th sympathetic ganglion of frog. The ganglion was exposed consecutively to five different drugs (concentration represented by solid line) while action potentials in the postganglionic nerve produced in response to electrical stimulation of the preganglionic nerve (stimulation rate, $\leqslant 1\ min^{-1}$) were monitored. Peak amplitudes of B waves (●) and C waves (○) were plotted as a function of time. Both B and C waves slowly declined over the 6.7 h time span shown. α-Conotoxin ImI (5 μM) almost completely blocked B waves (by 95%) but blocked C waves only slightly (by 21%). α-Conotoxin MI (5 μM) blocked B and C waves by only 26% and 25%, respectively. (+)-Tubocurarine ((+)Tc, 10 μM) almost completely blocked B waves (by 95%) but blocked C waves only slightly (by 26%). α-Conotoxin MII (5 μM) blocked B waves by 37% but blocked C waves nearly completely (by >94%). Dihydro-β-erythroidine (DβE, 5 μM) blocked both B and C waves almost completely (by 95% and >94%, respectively).

(compare Figure 3a and c). α-Conotoxin MI (5 μM) blocked B and C waves only minimally, but noticeably increased the latencies of both waves (see Figure 3b).

Discussion

In this study, three different *Conus* peptides were used, α-conotoxins MI, MII and ImI. All have structures with a disulphide-bonding framework typical of the α-conotoxin family (see Table 1). α-Conotoxin MI is a well-established blocker of skeletal muscle nicotinic AChRs (Olivera *et al*., 1985). α-Conotoxins MII and ImI have recently been shown to inhibit ACh-gated currents in *Xenopus* oocytes injected with mRNA encoding nicotinic AChRs from rat. Thus, α-conotoxin ImI preferentially inhibits α7 homomeric complexes expressed in *Xenopus* oocytes ($IC_{50} = 0.2$ μM), has lower potency for α9 homomeric complexes ($IC_{50} = 1.8$ μM), and has no effect on receptors composed of other combinations of subunits (Johnson *et al*., 1995). In contrast, α-conotoxin MII has highest affinity for the α3β2 combination of nAChR subunits ($IC_{50} = 0.5$ nM) and has at least two orders of magnitude lower potency on other combinations of nicotinic AChR subunits (Cartier *et al*., 1996). However, both α-conotoxins ImI and MII are capable of blocking the nicotinic acetylcholine receptor of frog skeletal muscle (McIntosh *et al*., 1994; Harris & Yoshikami, unpublished). Our working assumption is that these two α-conotoxins block synaptic transmission in frog ganglia by inhibiting nicotinic AChRs, although we have not rigorously ruled out other possibilities. The minimal activity of

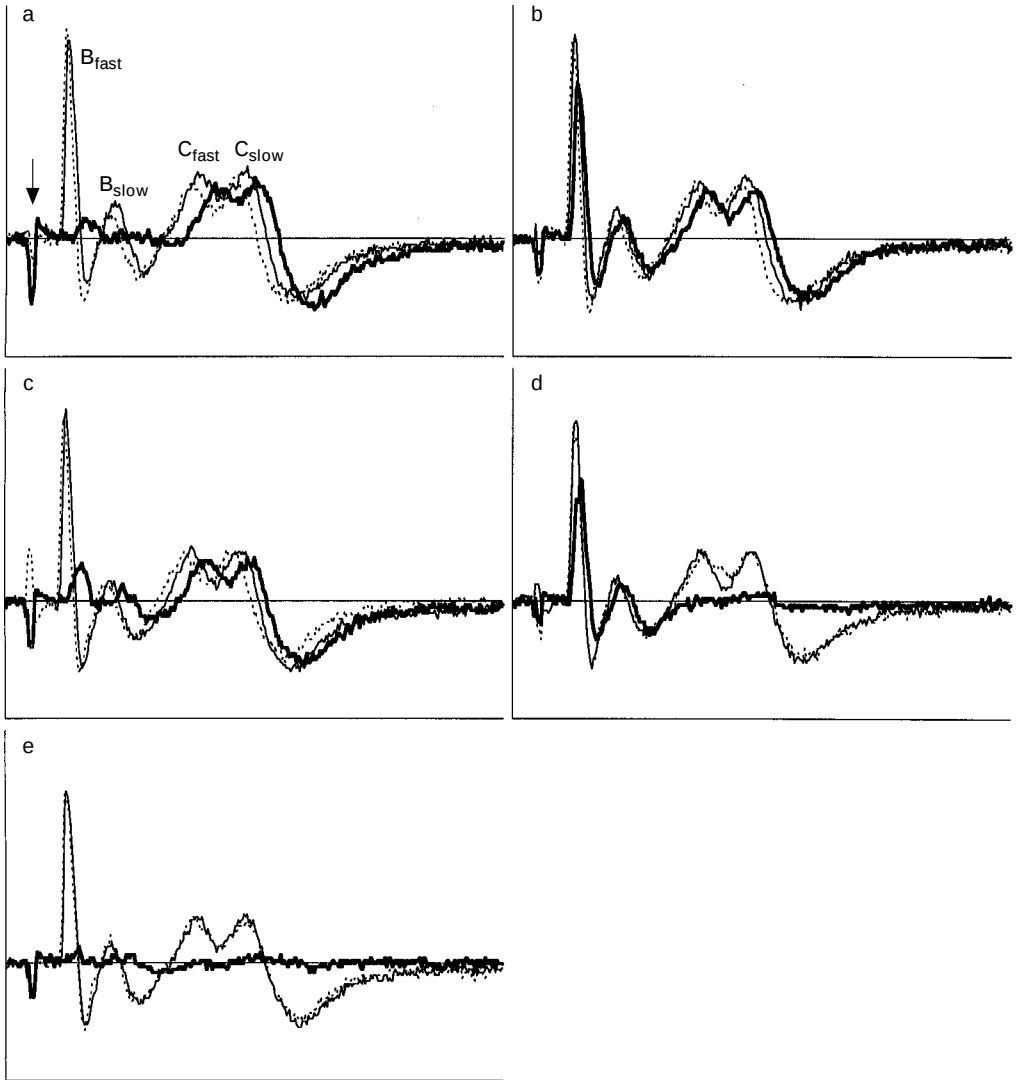

Figure 3 Traces of responses just before (thin solid curves), during (bold curves), and after (dashed curves), exposure to cholinergic antagonists. These responses were obtained during the experiment illustrated in Figure 2 and are presented in chronological order. The horizontal line in each panel represents the baseline (zero μV). The stimulus to the preganglionic nerve was presented 5 ms from the start of each trace (see arrow in first panel, note stimulus artifact at this location in all panels). In control responses (thin solid curve in each panel) the first deflection after the stimulus artifact is the fast B wave. It is followed by the smaller slow B wave then the double-humped C wave. (a) α-Conotoxin ImI (5 μM) was more effective in blocking B than C waves. Bold response was obtained 18 min after addition of 5 μM α-conotoxin ImI, and dashed response after 45 min of washing. (b) α-Conotoxin MI (5 μM) was not very effective in blocking either B or C waves. Bold response was obtained 55 min after addition of 5 μM α-conotoxin MI, and dashed response after 22 min of washing. (c) (+)-Tubocurarine (10 μM), like α-conotoxin ImI, was more effective in blocking B than C waves. Bold response was obtained 16 min after addition of 10 μM (+)-tubocurarine, and dashed response after 30 min of washing. (d) α-Conotoxin MII (5 μM), in contrast to α-conotoxin ImI and (+)-tubocurarine, was much more effective in blocking C than B waves. Bold response was obtained 14 min after addition of 5 μM α-conotoxin MII, and dashed response after 40 min of washing. (e) Dihydro-β-erythroidine (5 μM) blocked B and C waves equally well. Bold response was obtained 8 min after addition of 5 μM dihydro-β-erythroidine, and dashed response after 16 min of washing. In each panel, lengths of x- and y-axes are 120 ms and 120 μV, respectively.

α-conotoxin MI indicates that the skeletal muscle subtype of nicotinic AChR does not play a significant role in synaptic transmission in sympathetic ganglia, a conclusion consistent with the observation that synaptic transmission in frog sympathetic ganglia, unlike that in skeletal muscle, is not blocked by α-bungarotoxin (Shen *et al*., 1994). It might be noted that the sensitivities of a nicotinic AChR to various antagonists may be differentially altered by changes in the receptor ranging from a single amino acid substitution (e.g., Harvey & Leutje, 1996) to a post-translational modification such as glycosylation (e.g., Kreienkamp *et al*., 1994).

The simple method used in the present experiments of monitoring action potentials in the postganglionic nerve provides only an indirect assessment of the ability of an antagonist to block nicotinic AChRs. The apparent potency of a nicotinic antagonist will depend on the 'safety margin' of the excitatory synapse on the neurone; that is, how large the inward synaptic current is relative to that minimally necessary to reach threshold for generation of an action potential. Thus, if the safety margin of the synapses on B neurones were different from that of C neurones, a nicotinic antagonist would differentially block synaptic transmission through these two classes of neurones even if they possessed the same nicotinic AChRs. Indeed, Shen and Horn (1995) recently observed that in sympathetic ganglia of bullfrog the nicotinic synapses on B and C cells do have different safety margins; moreover, these safety margins were differentially use-dependent. Furthermore, by measuring synaptic currents under voltage-clamped conditions, Shen and Horn demonstrated that the nicotinic AChRs of B and C cells had identical sensitivities to (+)-tubocurarine. However, our experimental results cannot be explained simply by differences in safety margins because the nicotinic antagonists tested produced not only differential block of B vs C responses, but they did so with opposite selectivities. That is, whereas α-conotoxin ImI and (+)-tubocurarine blocked B responses more potently than C responses, α-conotoxin MII did just the reverse. These results strongly suggest that nicotinic AChRs of B neurones are pharmacologically distinct from those of C neurones.

The intrinsic safety margins of the nicotinic synapses of B and C neurones would not be expected to be static but instead be dynamically influenced by pre- and postsynaptic modulation such as depression and facilitation (e.g. Shen & Horn, 1995), long term potentiation (e.g. Minota *et al*., 1991), and the activities of homosynaptically activated muscarinic receptors as well as heterosynaptically activated peptidergic receptors (e.g. Jan & Jan, 1982; Kuffler & Sejnowski, 1983; Horn, 1992). Factors such as these may be reponsible for the variability we have observed in the sensitivities of responses to a given antagonist in different ganglia. Such variations from one ganglion to the next were also witnessed by Shen *et al*. (1994, see their Figure 2b) who examined the effects of neuronal bungarotoxin on nicotinic transmission in bullfrog sympathetic ganglia by an assay similar to ours. This discouraged us from attempting to obtain quantitative dose-response relationships for the α-conotoxins with the present extracellular assay. A more quantitative, albeit considerably more involved, assay would be to voltage-clamp neurones and monitor spontaneous miniature e.p.s.cs (e.g. Thigpen, 1995) or the responses to exogenously applied ACh (e.g. Akaike *et al*., 1989); this would provide a direct measure of the effect of an antagonist on AChRs. Use of such methods to characterize the effects of toxins remains for future experiments.

Our observations with (+)-tubocurarine are consistent with those of Nishi *et al*. (1965) who noted that in toad ganglia the orthodromic response of C neurones was more resistant to (+)-tubocurarine than was that of B neurones. In contrast, Shen and Horn (1995) observed the opposite in bullfrog ganglia stimulated at low frequency, i.e., the C, compared to B, wave is more sensitive to (+)-tubocurarine. The effects of the α-conotoxins on B and C neurones in ganglia of these other anuran species remain to be compared.

The various nicotinic antagonists did not discriminate between fast and slow B waves suggesting that the same subtype of nicotinic AChR may be shared by all B neurones. Likewise, the two components of the C wave were not differentially blocked by the antagonists, suggesting that the neurones responsible for the two-component C wave all have the same subtype of nicotinic AChR.

An increase in the latencies of partially blocked B and C waves was observed regardless of the antagonist producing the block (see Figure 3), and it is likely to be a reflection of an increased time required for the attenuated nicotinic e.p.s.p. to reach threshold to generate the action potential.

With regard to the two-component C wave, it is interesting to note that Nishi *et al*. (1965) observed that sympathetic axons of the sciatic nerve of toad (*Bufo vulgaris japonica*) had three distinct conduction velocities and classified them as B, C_1 and C_2 in view of their belief that C_1 and C_2 axons belong to C cells (but see Dodd & Horn, 1983). Perhaps C_1 and C_2 are the toad's counterpart of the two-component C waves we observed.

The prey of *C. magus*, whose venom contains α-conotoxin MII, is fish. Although α-conotoxin MII blocks skeletal nicotinic AChRs in frog (Harris & Yoshikami, unpublished), injection of α-conotoxin MII intramuscularly into goldfish does not cause paralysis (Cartier *et al*., 1996). This observation, in conjunction with the results presented here, tempts one to speculate that perhaps the role of α-conotoxin MII in *C. magus* venom might be to inhibit specifically the sympathetically mediated fight or flight response of fish prey, in contrast to the other toxins in *C. magus* venom, such as α-conotoxin MI and ω-conotoxin MVIIA, that paralyse fish by blocking neuromuscular transmission (Olivera *et al*., 1985). In this regard, it may be relevant that, as evident in Figures 2 and 3b, α-conotoxin MI appears to be relatively ineffective in blocking ganglionic nicotinic AChRs. It would be interesting indeed to compare the potencies of α-conotoxins MI and MII in blocking ganglionic nicotinic AChRs in fish.

The only other known peptidic ligand capable of blocking neuronal nicotinic AChRs such as those in frog sympathetic ganglia is neuronal-bungarotoxin (see Shen *et al*., 1994). Neuronal-bungarotoxin is a rather large peptide and unfortunately not readily available. In contrast, the α-conotoxins are relatively easily synthesized small peptides. In view of their availability, selectivity, and compact structures, we feel α-conotoxins will prove useful as reagents to probe further the structures and functions of the various neuronal nicotinic AChRs.

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