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Rank-order inhibition by ω-conotoxins in human and animal autonomic nerve preparations

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Abstract

The inhibitory effects of the ω-conotoxins GVIA, MVIIA and MVIIC on electrically-evoked, tetrodotoxin (10^{-7} M)-sensitive, autonomic nerve activity were studied using human, rat or guinea-pig vas deferens and intestinal tissues. In each preparation from each species, nM concentrations of ω-conotoxins GVIA and MVIIA prevented the neuronally-mediated contractions, whereas ω-conotoxin MVIIC was either markedly less potent (IC_{50}'s 1.4 or 2.9 log units more than for ω-conotoxin GVIA in guinea-pig ileum and rat vas deferens, respectively) or was without significant activity (human vas deferens, human *Taenia coli*) when tested at similar concentrations. In contrast the differences in potency between ω-conotoxins GVIA and MVIIC were considerably less when assayed directly on Ca^{2+} channel currents evoked from rat superior cervical ganglion neurons in culture (approximately 0.1 log unit difference) and from a stable cell line expressing rat α_{1B}, $\alpha_2\delta$, β_{1b} Ca^{2+} channel subunits (approximately 0.9 log unit). These different rank-orders of inhibitory activity of the conotoxins support the suggestion that there are pharmacologically distinct N-type Ca^{2+} channels in the peripheral nervous system, and that this tissue-dependent difference is seen in man.

Keywords: Ca^{2+} channel, N-type; ω-Conotoxin GVIA; ω-Conotoxin MVIIA; ω-Conotoxin MVIIC; Vas deferens; Intestine; Superior cervical ganglion; (Human); (Rat); (Guinea-pig)

1. Introduction

N-type calcium channels are specifically blocked by the ω-conotoxins, GVIA and MVIIA, and also less specifically, but potently, by ω-conotoxin-MVIIC (Spedding and Paoletti, 1992). Sensitivity of native sympathetic neuro-effector junctions to blockade by ω-conotoxins MVIIA and GVIA, coupled with characterization of Ca^{2+} currents recorded from sympathetic neurons in cell culture, has been used to define the presynaptic Ca^{2+} channels at sympathetic terminals as N-type (Olivera et al., 1994). The same toxins have been used to pharmacologically characterize the cloned recombinantly expressed Ca^{2+} channel subunits and show that N-type currents flow through channels containing the α_{1B} Ca^{2+} channel (Williams et al., 1992; Grantham et al., 1994).

α_{1B} Subunits can exist as a number of splice variants with specific localization patterns and these isoforms can be distinguished functionally (Lin et al., 1997, 1999). Further, in native systems there is evidence that there are pharmacologically distinct N-type channels. For one group of tissues, ω-conotoxin GVIA [= MVIIA] = MVIIC ≫ ω-Aga-IVA inhibitory rank order was reported against a noradrenergic-induced positive inotropic response in guinea-pig atria (Hong and Chang, 1995), rat hippocampal synaptic transmission (Wheeler et al., 1994), and electrically-induced activity in rat cerebellar granule cell neurites (Cousin et al., 1995). In contrast, in other tissue preparations, a rank-order inhibitory potency of ω-conotoxin GVIA [= MVIIA] ≫ MVIIC was reported against electrically-stimulated, neuronally-mediated contractions of rat vas deferens (Boot 1994; Wright and Angus 1996; Hirata et al., 1997; but not mouse vas deferens: Waterman, 1997), glutamate release from rat cortical synaptosomes (Turner and Dunlap, 1995), neurogenic relaxation of pig urethra (Werkstrom et al., 1995) and Ca^{2+} current in a hamster insulinoma cell line (Satin et al., 1995). Thus, in this

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second group of tissues ω-conotoxin MVIIC is surprisingly weak as a blocker appearing to define an ω-conotoxin MVIIA-sensitive but MVIIC-insensitive Ca^{2+} channel.

These differences in native tissue N-type channel pharmacology might be exploited in attempts to derive N-type channel blockers active within the central nervous system, without peripheral side effects. It was thus of interest to extend this work to human tissues. In this study, we have determined and then compared the rank-order inhibitory activities of the ω-conotoxins GVIA, MVIIA and MVIIC against electrically-stimulated, neuronally-mediated contractions of human and rat isolated vas deferens, and human and guinea-pig intestine. We confirmed the biological activity of the same batches of toxin by electrophysiological assay in a rat superior cervical ganglia preparation and a stable cell line expressing rat α_{1B}, $\alpha_2\delta$, β_{1b} subunits of the N-type calcium channel. Our results show that marked differences exist in the abilities of ω-conotoxins MVIIC and GVIA to inhibit neuronal activity and suggest that this difference is not necessarily species-dependent but may be tissue-dependent. The data are consistent with the idea that pharmacologically distinct N-type Ca^{2+} channels exist in the human peripheral nervous system. Preliminary data has previously been presented to the British Pharmacology Society (Ellis et al., 1996).

2. Materials and methods

2.1. Autonomic neuro-effector preparations

Tissues were suspended between two parallel platinum electrodes in tissue baths containing Kreb's (NaCl 121.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 25.0, glucose 5.6 mM) or Tyrode's (NaCl 136.9, $CaCl_2$ 2.2, KCl 2.7, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 12.1, glucose 5.6 mM) solution bubbled with 5% CO_2/95% O_2 and maintained at 37°C. In each case, electrical field stimulation evoked muscle contractions that were sensitive to inhibition by tetrodotoxin 10^{-7} M ($n = 4$ each) and hence, were mediated via stimulation of the intrinsic neurons. For all experiments the effects of the ω-conotoxin were measured once steady-state inhibition had been obtained; the times taken to achieve maximal activity varied according to the ω-conotoxin and tissue used. Time-matched solvent controls were performed in parallel, to assess the degree of fade in baseline contractile activity and to account for the reversible nature of the inhibition caused by ω-conotoxins MVIIA and MVIIC.

Data values are expressed as the maximum percentage inhibition by the ω-conotoxin of the electrically-evoked contraction amplitudes, when compared with the contraction amplitudes measured in the absence of ω-conotoxin. ω-Conotoxin potency (pIC_{50}; the concentration of ω-conotoxin which produced 50% of the maximal inhibition), was estimated by curve-fitting to the Hill equation.

2.1.1. Rat vas deferens

Whole vas deferens were removed from rats (Sprague–Dawley, 250–300 g) and mounted longitudinally in Kreb's solution under a load of 1 g for isotonic recording. These were stimulated continuously at 0.2 Hz with a biphasic square pulse width of 0.5 ms and maximal voltage (optimised to 60 V). Following an equilibration period, during which the tissues were flushed several times with fresh Kreb's solution and once the tissues had attained a consistent level of electrically-evoked contraction, cumulative concentration–effect curves were constructed to a single calcium channel blocker.

2.1.2. Human vas deferens

Human vas deferens were obtained with consent from patients undergoing elective vasectomies and stored in Tyrode's solution at 4°C for 12–24 h. These were dissected into 5 mm long sections and mounted in Tyrode's solution for measurement of circular muscle contraction under a resting tension of 1 g. Preparations were electrically stimulated for 1 s at 5 min intervals at a range of frequencies (usually 100 Hz) with a biphasic square pulse width of 0.5 ms at a voltage of 60 V. After obtaining electrically-evoked contractions with consistent amplitude, concentration–effect curves to a single calcium channel blocker were constructed by adding the blocker to the bathing solution after each period of electrical stimulation and subsequent washout of bathing solution.

2.1.3. Guinea-pig ileum

Segments of terminal ileum were removed from adult male guinea-pigs (350–450 g), cut into 1.5 cm long sections and longitudinal muscle-myenteric plexus preparations dissected as described previously (Sanger 1987). These preparations were suspended as described for the rat vas deferens, in Kreb's solution. These were stimulated continuously at 0.1 Hz with a biphasic square pulse width of 0.5 ms and a maximal voltage (optimised to 60 V) throughout the experiment. Following a 60 min equilibration period, concentration–effect curves to a single calcium channel blocker were as described for the rat vas deferens.

In some experiments, whole sections of ileum were used. These were suspended longitudinally and concentration–effect curves were constructed again using the rat vas deferens protocol.

2.1.4. Human Taenia coli

Macroscopically normal specimens of human *Taenia coli* were obtained from surgery for benign or malignant disease. These were used immediately or were stored overnight in oxygenated Kreb's solution at 4°C. After removal of the mucosa and submucosa, muscle strips

approximately 4 mm wide and 30 mm long were cut parallel to the longitudinal muscle fibres. These were suspended under a 1 g load in Kreb's solution. Tissues were stimulated for 30 s every 10 min at 5 Hz frequency and at maximum effective voltage. After obtaining consistent, electrically-evoked contractions, concentration–effect curves were constructed as described for the human vas deferens.

2.2. Electrophysiology

Rat superior cervical ganglion (SCG) cells were dissociated from male rats (100 g) using standard tissue culture techniques (Stansfeld and Mathie 1993) and recordings were made 2–7 days after isolation. Human embryonic kidney (HEK) 293 cells stably expressing α_{1B}, $\alpha_2\delta$, β_{1b} calcium channel subunits from rat were as described (Zamponi et al., 1997). Ba^{2+} currents through voltage operated calcium channels were recorded at room temperature (21–27°C), using the voltage clamp technique (Hamill et al., 1981) with a List EPC-7 amplifier, digitized and stored for off-line analysis using Cambridge Electronic Design software.

The internal (pipette) solution comprised (CsCl 140; HEPES 10; EGTA 10; $MgCl_2$ 4; ATP 2 mM, adjusted to pH 7.2 with CsOH). Modified Tyrode's bathing solution (NaCl 145; KCl 2.5; HEPES 10; Glucose 10; $CaCl_2$ 1.5; $MgCl_2$ 1.2 mM, adjusted to pH 7.4 with NaOH). Once whole cell recording was established the solution was changed to one allowing isolation of Ba^{2+} currents comprising ($BaCl_2$ 10; tetra-ethyl-ammonium [TEA] Cl 155; HEPES 10; Glucose 10; $MgCl_2$ 1 mM, adjusted to pH 7.4 with TEA OH). Ba^{2+} was used as the charge carrier to prevent calcium dependent inactivation and aid current isolation. Peak Ba^{2+} currents were evoked by depolarizing steps of 100 ms duration from -80 mV holding potential to a test potential of 0, $+10$ or $+20$ mV, repeated every 15 s. Nimodipine (10 μM) was added to the bathing solution and to all subsequent solutions applied to SCG cells in order to block L-type currents. Under these conditions, ω-conotoxin MVIIA and Nimodipine blocked 80.8% of the total current. In a separate set of experiments AgaIVA blocked $20.0 \pm 5\%$ ($n = 3$) of current consistent with most of the residual current being P/Q type. Thus under the assay conditions $> 80\%$ of the Ca^{2+} current was N-type. Solutions were applied by syringe and excess fluid removed by a suction tube allowing a rapid exchange of solutions (20 times bath volume within 10 s). Contact time of 3 min for each drug dose was used as a compromise between stabilization of block and current rundown.

2.3. Drugs

The ω-conotoxins GVIA, MVIIA and MVIIC were purchased from Bachem (UK) Ltd., and dissolved in water to give 100 uM stock solutions. AgaIVA was purchased from Alamone Labs Ltd., Israel. Tetrodotoxin and prazosin were purchased from Sigma-Aldrich Company Ltd. and

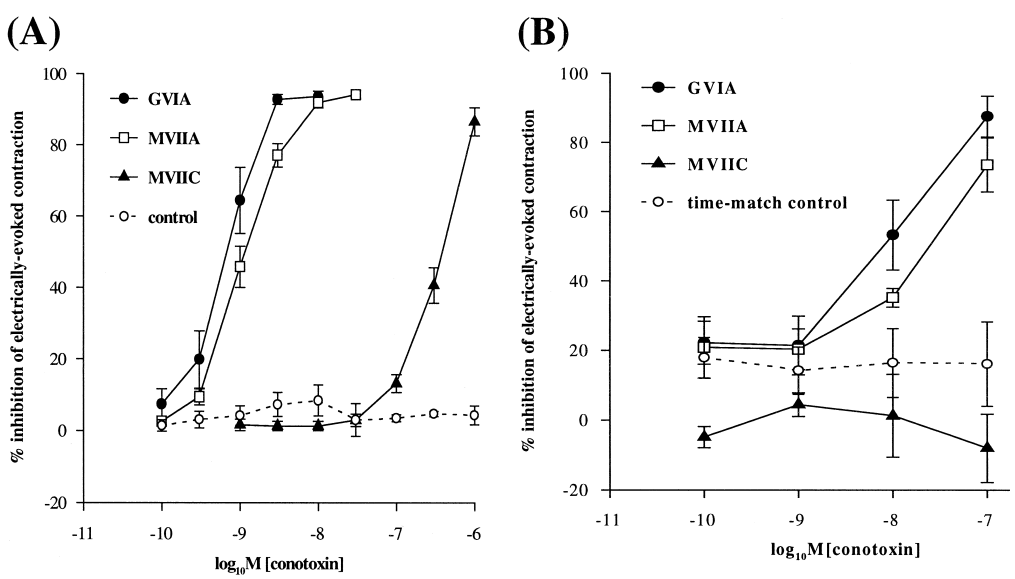

Fig. 1. Effects of ω-conotoxin GVIA (●), ω-conotoxin MVIIA (□), and ω-conotoxin MVIIC (▲) on electrically-stimulated, neuronally-mediated contractions of (A) rat and (B) human isolated vas deferens. Rat vas deferens were stimulated continuously at 0.2 Hz with a biphasic square pulse width of 0.5 ms and maximally-effective voltage (60 V). Human vas deferens were stimulated for 1 s at 5 min intervals at 100 Hz with a biphasic square pulse width of 0.5 ms at a voltage of 60 V. After obtaining consistent contractions, concentration–effect curves for a single ω-conotoxin were constructed by adding the toxin to the bathing solution either cumulatively (rat) or after the washout that followed each period of electrical stimulation (human). The effects of the ω-conotoxins were measured after maximal activity had been obtained. These times varied according to the conotoxin and tissue used (rat: 5 min for ω-conotoxins MVIIA and MVIIC, 30 min for ω-conotoxin GVIA; human 50–60 min for each); for the human vas deferens, small fluctuations in control contraction amplitudes during the long incubation time made it difficult to recognise small ω-conotoxin-induced changes in contraction amplitude. Time-matched solvent controls were performed in parallel (○). Points represent mean ± S.E.M; $n = 4$–7 each.

Table 1
Inhibitory effects of ω-conotoxins against electrically-evoked, neuronally-mediated contractions in human and animal nerve-smooth muscle preparations. All measurements were obtained after each ω-conotoxin had obtained equilibrium with the tissue. The effects of the ω-conotoxins are expressed in terms of their pIC_{50} values (the concentration which produced 50% of the maximal inhibition) and as the maximum percentage inhibition of the electrically-evoked contraction heights. Values are mean ± S.E.M

Conotoxin	Human vas deferens			Rat vas deferens		
	pIC_{50}	Max.% Inhibition	*n*	pIC_{50}	Max.% Inhibition	*n*
GVIA	8.1 ± 0.29	87.5 ± 5.9	5	9.2 ± 0.1	93.6 ± 1.5	7
MVIIA	7.5 ± 0.19	73.6 ± 7.8	6	9.0 ± 0.1	94.1 ± 1.2	5
MVIIC	Inactive ≤ 0.1 uM		7	6.3 ± 0.1	86.5 ± 4.0 at 1 uM	4
	Human *Taenia coli*			Guinea-pig ileum		
GVIA	7.5	82	1	8.1 ± 0.04	96.3 ± 0.3	6
MVIIA	8.4 ± 0.22	87.5 ± 3.3	4	8.2 ± 0.1	93.3 ± 1.4	4
MVIIC		24 ± 14 at 0.1 uM	3	6.7 ± 0.13	80.9 ± 5.7 at 1 uM	6

dissolved in water. Nimodipine was dissolved in ethanol and stock solutions were kept in the dark.

3. Results

3.1. Autonomic neuro-effector preparations

For each preparation, preliminary experiments were performed to determine the time required for each conotoxin to achieve a maximal effect and hence, equilibrium with the tissue. Obtaining data only when the toxins are at equilibrium with the tissue is critically important, since different toxins reach equilibrium at markedly different rates within the same tissue, or even for the same toxin tested in different tissues (Sather et al., 1993; Grantham et al., 1994; McDonough et al., 1996; Hirata et al., 1997). In the following experiments, cumulative concentration–effect curves were, therefore, constructed using variable contact times, to allow for these differences.

3.1.1. Rat vas deferens

The maximal inhibitory response to single concentrations of ω-conotoxins MVIIA and MVIIC were attained within 5 min, whereas ω-conotoxin GVIA required 30 min to achieve maximal activity. All three ω-conotoxins caused concentration-dependent inhibition of electrically evoked contractions (Fig. 1) with the rank order of ω-conotoxin GVIA > MVIIA ≫ MVIIC, based on pIC_{50} values (Table 1). The inhibitory activity of ω-conotoxin MVIIA and MVIIC, but not GVIA could be reversed by washing for 30 min.

3.1.2. Human vas deferens

Contact times of 50–60 min were necessary to achieve the maximum effects of each conotoxin and small fluctuations in control contraction amplitudes during this time (e.g., see Fig. 2) made it difficult to consistently detect small conotoxin-induced changes in contraction amplitude. Nevertheless, 10 and 100 nM ω-conotoxin GVIA and

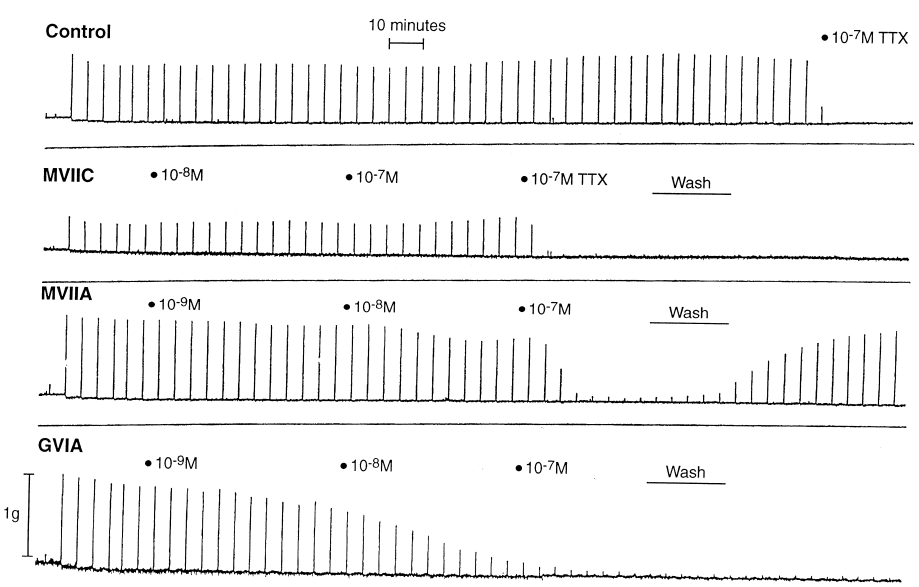

Fig. 2. Original records showing the effects of the ω-conotoxins GVIA, MVIIA and MVIIC on electrically-evoked contractions of human isolated vas deferens. The parameters of electrical stimulation are the same as for Fig. 1.

MVIIA clearly inhibited electrically-evoked contractions (Figs. 1 and 2), whereas ω-conotoxin MVIIC was without any consistent or concentration-dependent effect at concentrations up to and including 100 nM; pIC_{50} values and the maximal % inhibition attained with each conotoxin are given in Table 1. The inhibitory activity of ω-conotoxins MVIIA, but not GVIA, could be reversed by washing. Contractions were also completely abolished with prazosin 30 nM ($n = 4$). In one further experiment in which consistent electrically-evoked contractions were evoked using the lower frequency of 10 Hz, clear inhibitory activity was again obtained using 10 and 100 nM ω-conotoxins GVIA or MVIIA but not with 100 nM MVIIC (data not shown).

3.1.3. Guinea-pig ileum

The maximal inhibitory response to single concentrations of ω-conotoxin MVIIC was achieved within 5 min after addition to the bath, whereas contact times of 10 and 30 min were required for ω-conotoxins MVIIA and GVIA respectively. ω-Conotoxins GVIA and MVIIA were approximately equipotent and fully inhibited the electrically-evoked contractions, whereas ω-conotoxin MVIIC was only partially effective at the concentrations used (Table 1). The inhibitory activity of ω-conotoxins MVIIA and MVIIC, but not GVIA, could be reversed on washing.

The effects of ω-conotoxin MVIIC were also assessed in segments of whole terminal ileum to ascertain whether the lower potency of ω-conotoxin MVIIC observed in this study was due to the dissection of the preparation. Electrically-evoked contractions were obtained in whole ileum and in longitudinal muscle-myenteric plexus preparations removed from the same animals; compared with the dissected preparations, the electrically-evoked responses in the whole ileum were less consistent. Nevertheless, ω-conotoxin MVIIC caused similar, concentration-dependent inhibition of the contractions in segments of both whole and dissected ileum (pIC_{50} 7.12 ± 0.08 ($n = 6$) and 7.08 ± 0.1 ($n = 4$), respectively). Further, changing the frequency of stimulation from 0.05 to 0.2 Hz and the pulse width from 0.1 to 0.5 ms did not affect the potency of ω-conotoxin MVIIC relative to GVIA ($n = 3$, data not shown).

3.1.4. Human Taenia coli

ω-Conotoxin MVIIA 10^{-10} to 10^{-7} M caused concentration-dependent inhibition of contraction amplitude, maximal activity being achieved within 20 min after addition to the bathing solution. Similar inhibitory activity was also achieved with ω-conotoxin GVIA. By contrast, ω-conotoxin MVIIC 10^{-10} to 10^{-8} M had no consistent inhibitory activity, whereas 10^{-7} M caused a small reduction in contraction amplitude ($n = 3$; Table 1).

Table 2
Summary of interpolated pIC_{50} values for Ca^{2+} channel current block recorded from SCG cells and HEK 293 cells by ω-conotoxins MVIIA, MVIIC and GVIA
Values are mean ± S.E.M. ($n = > 4$ for each)

Cell type	GVIA	MVIIA	MVIIC
	pIC_{50}		
SCG	7.01 ± 0.09	7.49 ± 0.05	6.86 ± 0.12
HEK 293	7.71 ± 0.1	7.14 ± 0.07	6.75 ± 0.05

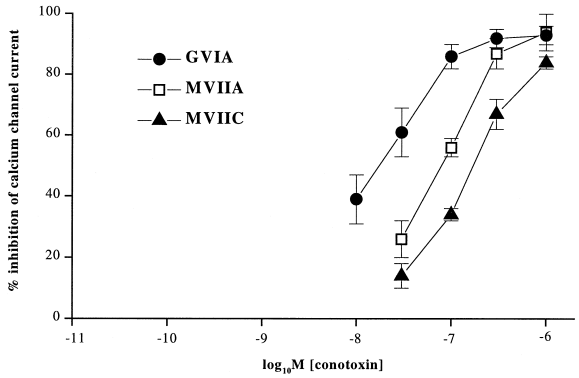

Fig. 3. Effects of ω-conotoxins GVIA (●), MVIIA (□) and MVIIC (▲) on Ba^{2+} currents recorded from HEK 293 cells stably expressing rat α_{1B}, $\alpha_2\delta$, β_{1b} Ca^{2+} channel subunits. Cells were recorded using the whole cell patch clamp technique. Peak activation currents were recorded following a 100-ms depolarizing step from −80 mV to 0, +10 or +20 mV. Nimodipine (10 μM) was bath-applied to block L-type current prior to ω-conotoxin application. Response for each dose of ω-conotoxin was taken after 3 min contact time. Points represent mean ± S.E.M; $n = 4$–5 each point.

3.2. Electrophysiology

Given the low potency/efficacy of ω-conotoxin MVIIC in the neuro-effector preparations, we tested the abilities of the ω-conotoxins to block calcium channel currents in rat SCG neurons and also on a recombinant HEK 293 cell line expressing rat α_{1B}, $\alpha_2\delta$, β_{1b} N-type calcium channel subunits (Zamponi et al., 1997). ω-Conotoxin was in contact with the cell for 3 min before measurements were made; this was sufficient time for block to stabilise at all but the lowest concentrations (McDonough et al., 1996). All three ω-conotoxins were potent inhibitors of Ba^{2+} current with less than 1 log unit difference in IC_{50} data (Table 2). Concentration–response curves for the ω-conotoxin actions on the recombinant cell line are shown in Fig. 3. These data confirm that each of the ω-conotoxins are approximately equi-potent inhibitors on both the well defined native N-type channel in SCG cells and a rat α_{1B}, $\alpha_2\delta$, β_{1b} channel expression system.

4. Discussion

In each of the human, rat and guinea-pig neuro-effector preparations, the ω-conotoxins GVIA and MVIIA potently and substantially inhibited the neuronally-mediated contractions. Whilst the absolute IC_{50} values varied between

tissues, most likely because of the necessary differences in the techniques used to evoke the contractions, the nM potencies of the ω-conotoxins (e.g., see Witcher et al., 1993), their activity kinetics (e.g., see De Luca et al., 1990) and their ability to cause near-maximal inhibition of the contractions are each consistent with a predominant role of N-type calcium channels in mediating the effects we have measured. This has not previously been shown using the human isolated tissues, but similar data has been described by others using rat vas deferens and guinea-pig ileum and similar parameters of electrical nerve stimulation (Boot 1994; Hirata et al., 1997; Tran and Boot 1997).

Again, in each of the human and animal nerve-muscle preparations, the inhibitory activity of ω-conotoxin MVIIC occurred at higher concentrations when compared with ω-conotoxins GVIA or MVIIA (IC_{50}'s 1.4 or 2.9 log units greater than for ω-conotoxin GVIA in guinea-pig ileum and rat vas deferens, respectively) or, at the concentrations tested, the toxin was without significant efficacy (human vas deferens, human *Taenia coli*). The relatively poor activity of ω-conotoxin MVIIC in guinea-pig ileum contrasts with the data of Boot (1994), who found that ω-conotoxins GVIA and MVIIC equipotently inhibited electrically-evoked contractions of intact preparations of guinea-pig ileum (pIC_{50}'s of 7.68 and 7.59 respectively). In our experiments, changing the method of tissue dissection or the parameters of electrical stimulation to match those used by Boot (1994) did not alter the low potency of ω-conotoxin MVIIC relative to ω-conotoxin GVIA. However later studies by Tran and Boot (1997) using guinea-pig ileum and the same experimental protocol as before, reported a lower potency for ω-conotoxin MVIIC relative to ω-conotoxin GVIA. The difference between the inhibitory activities of ω-conotoxins MVIIC and GVIA has also been reported using rat vas deferens (Boot 1994; Wright and Angus 1996; Hirata et al., 1997). However, similar data have not previously been obtained using the human isolated tissues and as such, our data indicates that the inhibitory rank order of ω-conotoxin GVIA = MVIIA ≫ MVIIC is both relevant to man and species-independent.

Conversely, in our experiments using rat superior cervical ganglia cells we observed no difference in either the potency or efficacy of ω-conotoxins GVIA or MVIIC. A small difference was observed using the stable cell-line expressing the rat α_{1B}, $\alpha_2\delta$, β_{1b} calcium channel subunits (0.9 log unit), and although this begins to approach the difference observed using guinea-pig isolated ileum (1.5 log unit), it remains substantially smaller than the large differences observed using these two toxins in any of the other neuro-effector preparations. Since, in superior cervical ganglia cells, the IC_{50} for ω-conotoxin MVIIC is similar to that in HEK 293 cells, it is unlikely that this value is contaminated significantly by block of the 20% residual non N-type current. Instead, the difference is likely to be largely explained by our use of 10 mM Ba^{2+} as charge carrier in these experiments, known to specifically reduce ω-conotoxin MVIIC potency on N-type channels (McDonough et al., 1996).

It is not clear what defines different rank-orders of ω-conotoxin activity in native tissues. Experimental conditions must clearly be a factor, especially the need to avoid large changes in divalent cation concentration and to ensure that measurements are taken when the toxin is in equilibrium with the tissue. Using rat vas deferens, for example, Hirata et al., (1997) demonstrated the sensitivity of the potency of ω-conotoxin MVIIC to divalent cation concentration, where increasing Ca^{2+} concentration from 1.5 to 5 mM significantly reduced the efficacy of 1 uM ω-conotoxin MVIIC, but not of ω-conotoxin GVIA. A further contribution could come from differences in the molecular structure of these channels. However, ω-conotoxin MVIIC has been shown to be as potent an inhibitor of channels containing α_{1B} pore forming subunit as ω-conotoxin GVIA for both human (Grantham et al., 1994) and rat isoforms (Fig. 3). Further attempts to identify heteromeric molecular species that are sensitive to ω-conotoxins MVIIA/ GVIA but not ω-conotoxin MVIIC have so far been unsuccessful (Meadows and Benham, 1999). Whatever the explanation, the need to recognize variations in rank-order inhibitory activities within native tissues is an important part of the process of characterizing such channels, especially if antagonists at these channels are to be designed for human use. Interestingly, the report of an apparent N-type calcium channel (defined by its electrophysiological characteristics) in rat dorsal raphe neurons, insensitive to inhibition by ω-conotoxin GVIA (Penington and Fox, 1995) further illustrates the need to continue this process.

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