ELSEVIER

European Journal of Pharmacology 451 (2002) 279–286

www.elsevier.com/locate/ejphar

Actions of intrathecal ω-conotoxins CVID, GVIA, MVIIA, and morphine in acute and neuropathic pain in the rat

David A. Scott *, Christine E. Wright, James A. Angus

Department of Pharmacology, University of Melbourne, Melbourne, Victoria 3010, Australia

Received 4 June 2002; received in revised form 7 August 2002; accepted 9 August 2002

Abstract

Agents which decrease conductance of N-type voltage-gated Ca^{2+} channels have been shown to attenuate measures of neuropathic pain in animal models and to provide symptom relief in humans. The ω-conotoxins have demonstrated efficacy but have a low therapeutic index. We have investigated the effects of a new ω-conotoxin, CVID (AM-336), and compared them with ω-conotoxin GVIA (SNX-124), ω-conotoxin MVIIA (SNX-111) and morphine in a spinal nerve ligation model of neuropathic pain in the rat. The ED_{50} (and 95% CI) for attenuation of tactile allodynia by intrathecal administration for ω-conotoxin CVID, GVIA, MVIIA and morphine was 0.36 (0.27–0.48), 0.12 (0.06–0.24), 0.32 (0.23–0.45) and 4.4 (2.9–6.5) μg/kg, respectively. Only morphine significantly prolonged acute tail flick responses (ED_{50} 2.3 (1.1–4.9) μg/kg). Of the ω-conotoxins, ω-conotoxin CVID showed the highest ratio of efficacy to behavioural toxicity. These observations show that intrathecal ω-conotoxins are effective in attenuating tactile allodynia in the rat without significantly affecting acute nociceptive responses. ω-Conotoxin CVID had similar potency to ω-conotoxin MVIIA but showed less toxicity in the therapeutic range.

Keywords: ω-Conotoxin; Morphine; Neuropathic pain; Ca^{2+} channel, N-type

1. Introduction

Neuropathic pain manifesting as allodynia and hyperalgesia continues to be a significant problem in clinical medicine. The neurophysiological mechanisms underlying the pathogenesis of these conditions are complex and, despite considerable research, are still incompletely understood. Morphine has been shown to be moderately effective in the treatment of chronic neuropathic pain, but not all individuals are responsive and in animals, tolerance develops to continued exposure (Levy et al., 1994; Malmberg and Yaksh, 1995). There is evidence that N-type voltage-gated Ca^{2+} channels (N-VGCCs) are involved in neurotransmitter release at a spinal cord level, and that blockade of these channels attenuates neuropathic pain responses (Chaplan et al., 1994b) and is less prone to the development of tolerance (Malmberg and Yaksh, 1995; Omote et al., 1996). ω-Conotoxin GVIA (ω-conotoxin GVIA, also known as SNX-124) is a peptide derived from the venom of the marine snail *Conus geographus* which blocks N-type Ca^{2+} channels in a highly selective manner (Plummer et al., 1989). The potency in vivo of ω-conotoxin GVIA is greater than that of the related peptide from *Conus magus*, ω-conotoxin MVIIA (SNX-111) (Vega et al., 1995). ω-Conotoxin MVIIA has been shown to inhibit neuropathic pain responses using the spinal (intrathecal) route in animal studies (Bowersox et al., 1996), and it has also been used in clinical trials (Brose et al., 1997). Animal studies suggest that ω-conotoxin MVIIA has higher potency and induces less tolerance than morphine (Bowersox et al., 1996; Malmberg and Yaksh, 1995). However, side effects have been reported with clinical use of ω-conotoxin MVIIA (Penn and Paice, 2000), so there is a need to investigate alternatives.

In this study, we compared the antinociceptive activity of intrathecal morphine, ω-conotoxin GVIA, and ω-conotoxin MVIIA with a new ω-conotoxin from *Conus catus*, CVID (AM-336) (Lewis et al., 2000; Nielsen et al., 2000), in a rat model of neuropathic pain.

2. Materials and methods

Male Sprague–Dawley rats were housed in a facility with a 12-h day–night cycle and free access to food and water. They were caged in groups until after intrathecal catheter

* Corresponding author. Tel.: +61-3-9288-2211; fax: +61-3-9288-4193.
E-mail address: scottdav@bigpond.net.au (D.A. Scott).

0014-2999/02/$ - see front matter © 2002 Elsevier Science B.V. All rights reserved.
PII: S0014-2999(02)02247-1

placement, when they were caged separately but adjacent to other animals. Animals were observed regularly throughout the treatment period and any showing signs of distress were removed from the study and humanely killed. The sequence of the study is shown in Table 1. All experiments were approved by the University of Melbourne Animal Research Ethics Committee.

2.1. Neuropathy

Production of a unilateral hind-limb neuropathy was achieved using the technique of spinal nerve ligation (Kim and Chung, 1992) on rats weighing 140–160 g. Briefly, following gaseous induction and maintenance of anaesthesia with halothane (4% decreasing to 1.5%) in oxygen, a dorsal paramedian incision was made to the left of the vertebral column at the level of the L4 to L6 vertebrae. Deep dissection, including partial resection of the transverse process of L6 allowed the L5 and L6 spinal nerves to be identified and isolated distal to the dorsal root ganglion. At this point, they were tightly ligated but not transected, using 6–0 silk sutures. The wound was then closed in layers and the rats allowed to recover in a warm box. A small number of animals had sham surgical procedures, identical to the above but excluding manipulation or ligation of the spinal nerves.

Animals were given a week to recover from surgery before testing to confirm development of neuropathy (Table 1).

2.2. Intrathecal catheters

Animals were assessed for neuropathy every second day commencing 1 week following nerve ligation. Once von Frey thresholds (see below) of less than 10 g had been measured on two consecutive testing occasions, chronic intrathecal catheters were implanted, this was typically 14 days following nerve ligation. A modification of the technique described by Yaksh and Rudy (1976) was used. Following gaseous induction and maintenance of anaesthesia with halothane (4% decreasing to 1.5%) in oxygen, the animal was supported in a stereotaxic frame and the atlanto-occipital membrane was exposed using aseptic techniques. A 32-gauge polyimide catheter was then threaded into the subarachnoid space and passed in a caudal direction for approximately 8 cm, to end in the region of the lumbar expansion. The external end of the catheter was connected to a length of PE-10 polyethylene tubing which was tunnelled and exteriorised over the forehead. The skin wound was closed and the exteriorised catheter end was plugged after flushing with 10 μl of saline, catheter dead space being approximately 7 μl. Animals were then caged individually and allowed to recover for 3 days before treatment. Any animal developing motor impairment following catheter placement was excluded from the study.

Animals that had sham neuropathy surgery performed had intrathecal catheters placed and contributed to the measurement of tail-flick response and the observation of toxicological effects.

Table 1
Study sequence

Day	Procedure
0	Baseline testing
0	Neuropathy surgery
6	Testing
9	Testing[a]
	Intrathecal catheter placement
12	Testing
	Agent Administration
	Testing at 0.5 h
	Testing at 1 h
	Testing at 2 h
13	Testing at 24 h
14	Testing at 48 h
	Confirmation of catheter function

[a] Only animals with successful neuropathy surgery or sham surgery proceeded past this point.

2.3. Drugs

Drugs were dissolved in sterile saline and administered as a bolus via the intrathecal catheter in volumes of 9–13 μl. The catheter was flushed afterwards with 10 μl saline. Control animals received 10 μl saline only. ω-Conotoxin GVIA was delivered in doses of 0.01, 0.03, 0.06, 0.1, 0.3 and 1.0 μg/kg. ω-Conotoxin MVIIA and ω-conotoxin CVID were delivered in doses of 0.03, 0.1, 0.3, 1.0 and 3.0 μg/kg, and ω-conotoxin CVID also at 10 μg/kg. Morphine was administered in doses of 0.1, 1.0, 3.0, 10.0 and 100.0 μg/kg. All peptide doses were adjusted for the percentage of active peptide present in the source powder. ω-Conotoxin GVIA was synthesized by the Centre for Drug Design and Development, The University of Queensland (St. Lucia, Qld, Australia). ω-Conotoxin CVID and MVIIA were provided by AMRAD (Burnley, Victoria, Australia).

2.4. Testing

Testing of the animals involved measuring their responses to acute pain using heat (tail flick) and to neuropathic pain (tactile allodynia) using touch (von Frey hairs). In addition they were regularly weighed. All testing was done after the animals had familiarized themselves with their enclosures (usually 10 to 15 min) and were resting quietly. For tail-flick testing, animals were housed in an enclosure with a glass floor, and allowed to settle. In this way, the animals could be tested without physical restraint. A focused infra-red heat source with an in-built timer (Plantar Test, Ugo Basile, Italy) was then directed under the tail at least 5 cm from the base, and the time was automatically measured until the tail was withdrawn, which was usually with a characteristic twitch. The heat source was adjusted to a level which resulted in a baseline latency

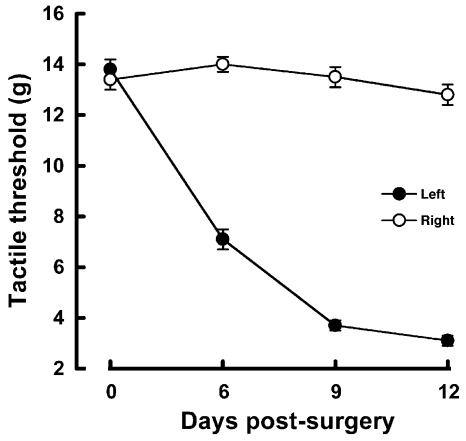

Fig. 1. Tactile allodynia as measured by withdrawal threshold (g) to von Frey hairs applied to the left (operated) and right (non-operated) hind paw. Data points are mean ± S.E. $n = 116$.

of approximately 3 s. This required an upper limit of stimulus duration to be set at 10 s to prevent thermal injury. Three measurements were taken for each animal, with at least 60-s rest between readings. The average of these three values was taken to be the tail-flick latency, with 10 s as the maximum possible value.

The degree of neuropathic pain was determined by measuring the level of tactile allodynia. Following nerve ligation surgery, the hind paw on the operated side became sensitive to normally innocuous levels of touch, evoking a withdrawal response at a much lower pressure threshold (tactile allodynia). von Frey hairs were used to measure the withdrawal pressure threshold (Stoelting, IL, USA) (Chaplan et al., 1994a).

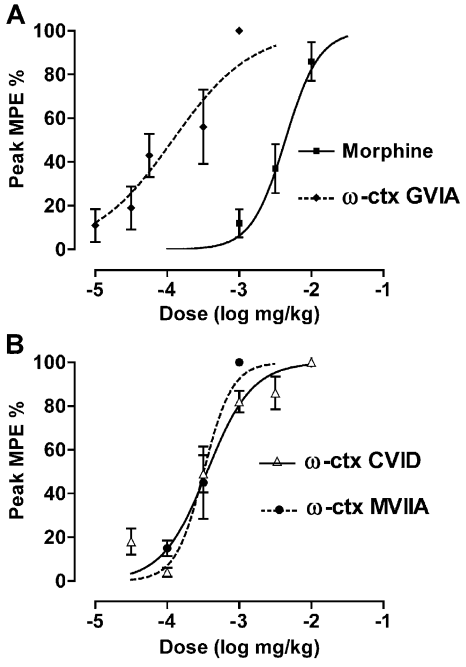

Fig. 2. Log dose–response curves for (A) intrathecal morphine ($n = 28$) and ω-conotoxins GVIA ($n = 22$) and (B) MVIIA ($n = 17$) and CVID ($n = 41$) in reduction of tactile allodynia. Outcome expressed as percentage of maximal possible effect (%MPE). Data points are mean ± S.E.

The test animals rested in a clear enclosure, the base of which was an open wire mesh such that the plantar surface of the paw was easily accessible from below. The hairs were applied sequentially, starting with 2 g of force and proceeding upwards to a maximum of 15.1 g force or downwards to a minimum of 0.4 g force. A force of 15.1 g force thus defines the 'maximum possible effect' (MPE) for this test and chosen so as to be just short of enough force to physically lift the hind paw off the mesh. Once a paw withdrawal occurred, the 50% withdrawal threshold was determined using the 'Up–down' method as published by Dixon (1980). Both hind paws were tested.

Toxicity was assessed by behavioural observation and rated as nil, mild, moderate or severe. Mild toxicity manifests as occasional spontaneous tail twitching or lower back hunching and piloerection. Moderate toxicity showed as frequent tail flicks or occasional writhing of the tail and spontaneous hind-limb twitching accompanied by ataxia. Severe toxicity was described when marked tail writhing occurred along with frequent hind-limb twitching or hind-limb splaying and an inability to walk normally.

At the conclusion of the treatment cycle, the correct placement of the intrathecal catheters was confirmed by an

Table 2
Summary of potencies of intrathecal administration of ω-conotoxins and morphine to attenuate tactile allodynia and to demonstrate moderate or severe toxicity

	ω-Conotoxin GVIA (μg/kg)	ω-Conotoxin MVIIA (μg/kg)	ω-Conotoxin CVID (μg/kg)	Morphine (μg/kg)
ED_{50} (μg/kg) (*n*)	0.12 (22)	0.32 (17)	0.36 (41)	4.36 (28)
C.I.	(0.06–0.24)	(0.23–0.45)	(0.27–0.48)	(2.9–6.5)
Relative potency (a) to Morphine	36	13.6	12.1	1
(b) to CVID	3.0	1.1	1	
TD_{50} (μg/kg) (n)	0.60 (33)	0.68 (21)	3.5 (48)	
C.I.	(0.56–0.65)	(0.26–1.8)	(3.1–4.0)	
Relative toxicity	5.8	5.1	1	
Therapeutic index (TD_{50}/ED_{50})	5.0	2.1	9.7	

Values expressed with 95% CI.
ED_{50} is dose of agent (μg/kg) causing 50% MPE in tactile allodynia attenuation. Relative potency (a) is the ED_{50} of the peptides compared with morphine, and (b) is the ED_{50} of the ω-conotoxins GVIA and MVIIA compared with CVID. TD_{50} is dose of agent causing moderate to severe behavioural toxicity. Relative toxicity is the TD_{50} of the ω-conotoxins GVIA and MVIIA compared with CVID. (*n*) is number of rats—note that for TD_{50}, *n* includes sham neuropathy animals.

intrathecal injection of 10 μl of lidocaine (2%). Observation of symmetric paralysis confined to the hind limbs was considered to indicate satisfactory positioning and patency of the catheter. Animals were not included in the study if a satisfactory block was unable to be demonstrated.

2.5. Data analysis

Data was analysed using analysis of variance (ANOVA) for continuous variables, with repeated measure adjustment where appropriate. Contingency table analysis was used for nonparametric data. Values are expressed as mean ± standard error (S.E.), standard deviation (S.D.) or 95% confidence intervals. Response to therapy as measured by tactile allodynia threshold or tail-flick latency was calculated using the percentage maximum possible effect (%MPE) using the following equation: %MPE=([Measured Value] − [Pretreatment value]) × 100/([MPE] − [Pre-[Pretreatment value]). Sigmoidal nonlinear regression curve fitting for dose–response data and estimation of ED_{50} and TD_{50} was done using GraphPad Prism 3 software (GraphPad Software, San Diego, CA, USA). A *P*-value of less than 0.05 was taken to indicate statistical significance.

3. Results

3.1. Establishment of neuropathy

Following nerve ligation surgery, the tactile allodynia thresholds were significantly lower in the left (operated) hind paw compared with the right ($P < 0.001$, repeated measures ANOVA, $n = 116$). The mean (± S.E.) threshold for the left hind paw prior to agent administration was 3.0 (± 0.2) g, compared with 12.5 (± 0.4) for the right side (Fig. 1). There were no significant effects on tail-flick thermal latency following nerve ligation.

Sham surgery was performed in 20 animals. There was no significant difference between ipsilateral and contralateral hind limbs at any time during testing (data not shown).

3.2. Dose–response data

The peak effect of each dose of drug in attenuating tactile allodynia is plotted in Fig. 2 as dose–response curves and the estimated effective doses for a 50% MPE (ED_{50}) are in Table 2. For morphine, the time to peak response with allodynia was 30–60 min whereas for the conotoxins this occurred later at 120 min. From these data, it is clear that

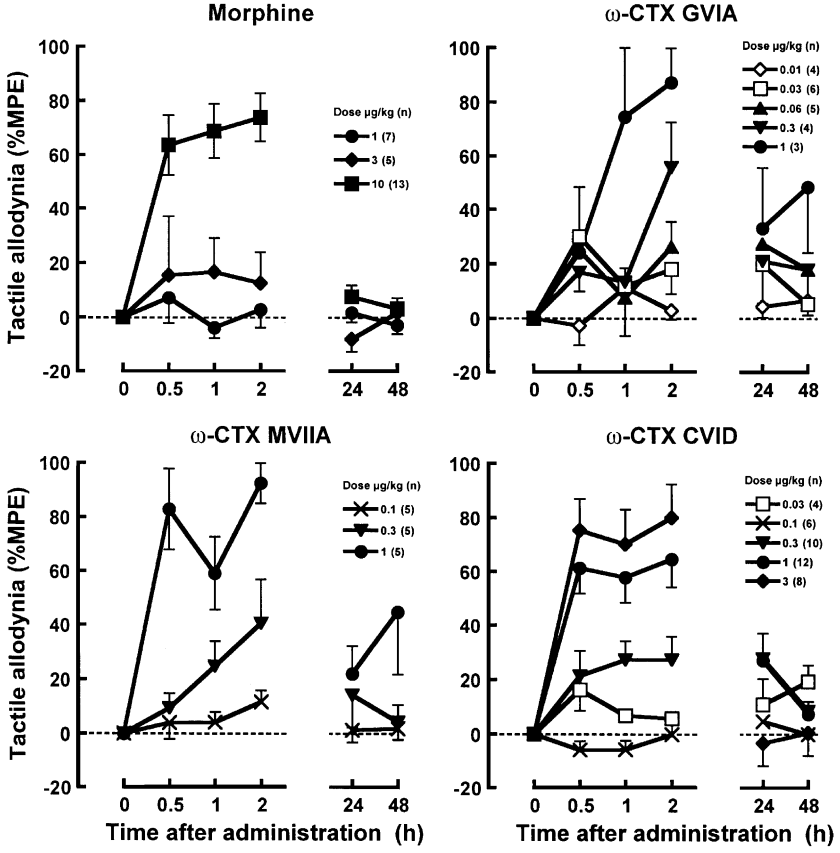

Fig. 3. Tactile allodynia reduction over time expressed as percentage of maximal possible effect (%MPE) (μg/kg IT). Data is shown only for treatment groups containing more than two animals. ω-ctx = ω-conotoxin.

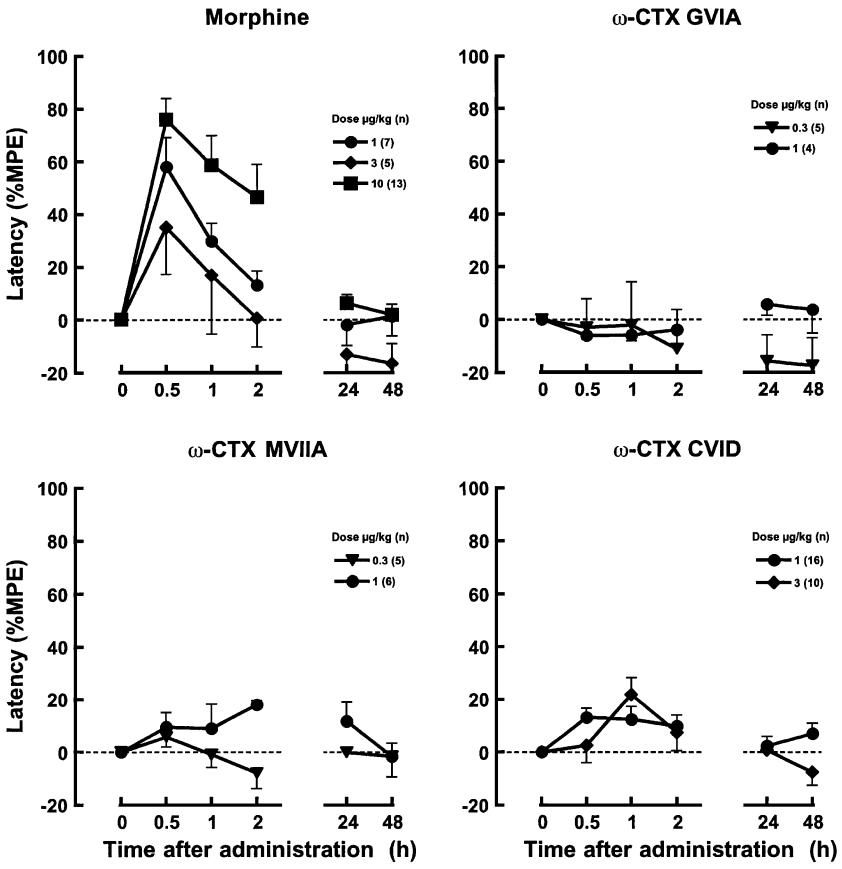

Fig. 4. Tail-flick latency prolongation expressed as percentage of maximal possible effect (%MPE) (µg/kg IT). Data is shown only for treatment groups containing more than two animals. ω-ctx = ω-conotoxin.

ω-conotoxin MVIIA and ω-conotoxin CVID have similar potency, and that ω-conotoxin GVIA is significantly more potent than ω-conotoxin CVID ($P < 0.05$). None of the agents tested had any measurable effect on the contralateral hind limb (data not shown). Dose limiting side effects of morphine and ω-conotoxin MVIIA prevented a full dose–response curve being produced with these agents (see below).

Residual effects on tactile allodynia at 24 h after administration were not detectable with morphine. There was some evidence for a weak residual effect at 24 to 48 h with the ω-conotoxins (Fig. 3), especially at higher doses.

Baseline tail flick latency was 3.3 s overall, and did not differ between groups. The ω-conotoxins had no discernable effect on tail-flick latency. Morphine, however, had an ED_{50} of 2.3 µg/kg for prolonging tail-flick latency (95% CI 1.1–4.9 µg/kg). This effect peaked at 30 min (Fig. 4).

3.3. Toxicity

The proportion of animals in each ω-conotoxin treatment group manifesting signs of moderate or severe toxicity is shown in Fig. 5. The onset of toxicity, when it occurred, was always within 30 to 60 min. Mild toxicity was usually gone by 2 h and toxicity of all grades was gone by 24 h. Severe toxicity frequently precluded accurate assessment of allodynia or tail-flick threshold due to spontaneous movements. Based on these data, the estimated dose for 50% of animals to show moderate or severe toxicity (TD_{50}) is shown in Table 2. Toxic behaviour

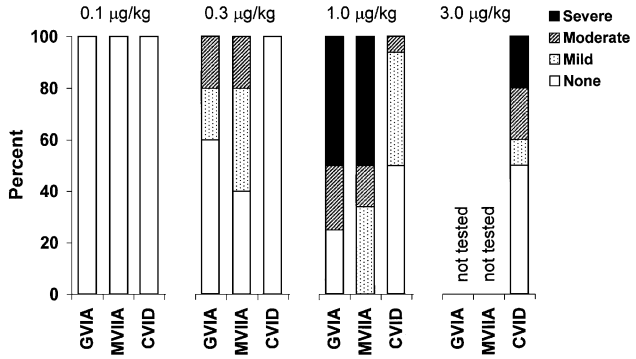

Fig. 5. The percentage of animals manifesting toxic behaviour receiving ω-conotoxins GVIA, MVIIA and CVID at four IT dose levels. Toxicity levels: none (clear □), mild (speckled ⊡) moderate (cross-hatched bars ▨) or severe (filled bars ■).

occurred at lower doses of ω-conotoxin MVIIA than for ω-conotoxin CVID. For the ω-conotoxins, the ratio of ED_{50} to TD_{50} was greatest for ω-conotoxin CVID and least for ω-conotoxin MVIIA (Table 2).

Morphine did not show any of the characteristic toxic manifestations found with the conotoxin-treated animals. However, a number of animals did not withdraw from the tail-flick heat source within the time-out period of 10 s, and might therefore be presumed to have lost noci-protective responses. This occurred in 20% of animals at 1 μg/kg, 40% at 3 μg/kg, 70% at 10 μg/kg and 100% at 100 μg/kg doses. These animals also showed a reduction in spontaneous motor activity. At the highest dose (100 μg/kg), the animals were stuporose, presumably due to spread to the brain, and so data for allodynia and tail-flick responses were not used.

3.4. Weight

There were no differences between groups in body weight during the course of the study. At the time of agent administration, the overall mean weight ($\pm$ S.D.) was 209 ± 14 g.

4. Discussion

This study demonstrates that the intrathecal administration of morphine and ω-conotoxins GVIA, MVIIA and CVID is effective in attenuating neuropathic pain in the rat. From the ED_{50} values calculated at 50% MPE, ω-conotoxin GVIA was three to four times more potent than ω-conotoxin MVIIA and ω-conotoxin CVID, and approximately 40 times more potent than morphine. Importantly, morphine but not the conotoxins, also prolonged tail-flick latency. Amongst the ω-conotoxins, CVID had the largest ratio of ED_{50} to TD_{50}, thus potentially providing a greater therapeutic margin.

The ED_{50} with intrathecal ω-conotoxin MVIIA has been reported to range from 32 to 400 ng/kg in rats for suppressing flinches in response to plantar formalin injection (Bowersox et al., 1996; Malmberg and Yaksh, 1994). Data are often reported on a total dose rather than dose by body weight basis, however when data for animal weights was provided by authors, values were able to be estimated for comparison with the present work. In animals with tactile allodynia, the ED_{50} for intrathecal ω-conotoxin MVIIA was estimated to be approximately 200 ng/kg (Bowersox et al., 1996) which is consistent with our findings with ω-conotoxin MVIIA (ED_{50} 300 ng/kg, 95% CI 160–560 ng/kg).

Behavioural toxicity was observed by Malmberg and Yaksh (1994) in some animals with bolus doses of ω-conotoxin MVIIA in a similar range of doses as for the antinociceptive effects, and in a large proportion of animals receiving high-dose chronic intrathecal infusions (Malmberg and Yaksh, 1995). An intrathecal dose of 3 μg ω-conotoxin MVIIA (estimated 15 μg/kg) has been reported to cause moderate motor dysfunction in 100% of rats (Chaplan et al., 1994b). This dose is approximately 18 fold higher than doses tolerated by our animals, however the ED_{50} for suppression of tactile allodynia was approximately 2 μg (estimated 10 μg/kg) compared with our estimate of 0.32 μg/kg. Although the difference in absolute potency cannot be readily explained, the therapeutic index of 1.5 is similar to our estimate of 2.1.

The responsiveness of neuropathic pain to morphine is variable. Although found by some to be effective (Suzuki et al., 1999), others have demonstrated minimal benefits (Catheline et al., 2001). This is consistent with human experience (Portenoy et al., 1990) and may relate to the pathogenesis of the pain. Morphine administered by the intrathecal route appears to be the most promising (Suzuki et al., 1999) although tolerance develops by whichever route is used (Malmberg and Yaksh, 1995). We found morphine administered by the intrathecal route was not only effective in attenuating allodynia but was also associated with stupor when using the higher doses, presumably as a result of spread to the central nervous system.

The lack of a significant effect of spinal injection of conotoxins on thermal tail-flick responses is consistent with other reports. Malmberg and Yaksh (1994) found only 20% MPE effect on hot-plate responses using an intrathecal bolus dose of ω-conotoxin MVIIA which caused maximal inhibition of formalin responses. We found morphine to prolong tail-flick latencies. The ED_{50} for intrathecal morphine may vary according to stimulus site and intensity (Abram et al., 1997). The high intensity thermal plantar test in Abram et al.'s (1997) study most closely matched the baseline latency of tail flick in our study and provided an ED_{50} estimate for intrathecal morphine of 1.6 μg/kg (95% CI 0.8–3.1 μg/kg). This is not significantly different from our own estimate of 4.4 μg/kg (95% CI 2.9–6.5 μg/kg). However, Omote et al. (1996) described a higher ED_{50} for intrathecal morphine of 5.1 μg (estimated 15 μg/kg) using the tail-flick response. One possible explanation for this is from electrophysiological studies which have shown that depression of dorsal horn neuronal responses with intrathecal morphine is achieved with lower doses in rats with spinal nerve ligation neuropathy compared with sham operated rats (Suzuki et al., 1999). However, clinical experience is of inconsistent and limited effectiveness of opioids with neuropathic pain.

ω-Conotoxins are peptides derived from the venom of the marine sea snail which share the property of selective blockade of mammalian N-VGCCs. The influx of Ca^{2+} through this channel facilitates the presynaptic release of neurotransmitters which have been shown to be involved with nociceptive processing. ω-Conotoxins have similar folded structures (ω-conotoxin CVID being more tightly folded than ω-conotoxin GVIA or ω-conotoxin MVIIA (Lewis et al., 2000; Nielsen et al., 2000)) and reasons for differences in potency or toxicity are unclear. ω-Conotoxin GVIA has a high affinity for the channel and dissociates more slowly than ω-conotoxin MVIIA (Hirata et al., 1997).

Comparative functional studies yield different results depending on the tissue used, and there is evidence that different sub-types of N-VGCC may exist (Sanger et al., 2000; Wright et al., 2000). Greater selectivity for neuronal N-VGCC over P/Q type Ca^{2+} channels has been demonstrated for ω-conotoxin CVID over ω-conotoxin MVIIA in both radioligand binding studies and in influencing Ca^{2+} conductance in rat N-VGCC (Lewis et al., 2000). These properties suggested ω-conotoxin CVID as a potentially useful agent for in vivo evaluation. The cardiovascular and autonomic reflex effects of ω-conotoxin CVID and ω-conotoxin MVIIA were compared in conscious rabbits using intravenous and intrathecal routes of administration (Wright et al., 2000). In this study, there were no cardiovascular effects following intrathecal administration. In contrast, after intravenous administration, similar vagolytic and sympatholytic effects on the baroreflex occurred with both peptides (Wright et al., 2000). There was a difference, however, in the orthostatic response, with the postural hypotension induced by intravenous ω-conotoxin MVIIA lasting for over 90 min compared with only 30 min for ω-conotoxin CVID. This finding was consistent with lower potency in in vitro responses to rat isolated mesenteric artery constriction following perivascular nerve stimulation with ω-conotoxin CVID compared with ω-conotoxin MVIIA (Wright et al., 2000).

In the central nervous system, μ-opioid receptors are coupled to N-VGCCs by a G-protein mechanism (Rhim and Miller, 1994) and stimulation results in inhibition of neurotransmitter release. The finding that ω-conotoxin selectively attenuates tactile allodynia in this rat preparation of neuropathic pain, whilst having no effect on acute nociceptive responses (tail-flick withdrawal to heat), suggests that morphine has sites of action in addition to those associated with N-VGCCs. Although morphine acts at both supraspinal and spinal sites, behavioural effects were only seen with the highest doses, so it is likely that the effects seen were predominantly spinal. Such additional sites could include the postsynaptic membrane, where opioids also act to hyperpolarize the postsynaptic neuron by increasing potassium conductance.

If the finding of a separation of analgesia modalities of ω-conotoxins translates into humans then these agents may prove superior to the less selective analgesics in the treatment of certain types of neuropathic pain. However, early clinical experience with ω-conotoxin MVIIA demonstrates that side effects may limit the usefulness of this class of drug (Atanassoff et al., 2000; Wang et al., 2000). A phase I/II clinical trial is currently underway to see whether ω-conotoxin CVID is better tolerated in humans in line with the greater therapeutic index seen in this rat study, although side effects may still be expected at the upper dose range. Further investigation into the combination of ω-conotoxins with other analgesics such as morphine also needs to be undertaken because synergistic effects would enable lower doses of each agent to be used, thus limiting toxic side effects. At present, however, the utility of this class of drug is limited by the need for spinal administration.

In conclusion, this study demonstrates that intrathecal ω-conotoxins GVIA, MVIIA and CVID are effective in reducing tactile allodynia in the rat. ω-Conotoxin GVIA is more potent than the other two peptides but ω-conotoxin CVID shows the highest ratio of efficacy to toxicity. These compounds were selective in that they did not significantly attenuate acute thermal nociception. Intrathecal morphine was also effective in decreasing tactile allodynia but was less potent and less selective, as it attenuated acute thermal nociception as well.

Acknowledgements

We gratefully acknowledge the Australian and New Zealand College of Anaesthetists for a research grant which supported part of this project. Conotoxin compounds and a grant in aid were supplied by AMRAD (Burnley, Victoria, Australia). We thank Linda Cornthwaite-Duncan for her valuable assistance.

References

Abram, S.E., Mampilly, G.A., Milosavljevic, D., 1997. Assessment of the potency and intrinsic activity of systemic versus intrathecal opioids in rats. Anesthesiology 87, 127.

Atanassoff, P.G., Hartmannsgruber, M.W., Thrasher, J., Wermeling, D., Longton, W., Gaeta, R., Singh, T., Mayo, M., McGuire, D., Luther, R.R., 2000. Ziconotide, a new N-type calcium channel blocker, administered intrathecally for acute postoperative pain. Reg. Anesth. Pain Med. 25, 274.

Bowersox, S.S., Gadbois, T., Singh, T., Pettus, M., Wang, Y.X., Luther, R.R., 1996. Selective N-type neuronal voltage-sensitive calcium channel blocker, SNX-111, produces spinal antinociception in rat models of acute, persistent and neuropathic pain. J. Pharmacol. Exp. Ther. 279, 1243.

Brose, W.G., Gutlove, D.P., Luther, R.R., Bowersox, S.S., McGuire, D., 1997. Use of intrathecal SNX-111, a novel, N-type, voltage-sensitive, calcium channel blocker, in the management of intractable brachial plexus avulsion pain. Clin. J. Pain 13, 256.

Catheline, G., Le Guen, S., Besson, J.M., 2001. Intravenous morphine does not modify dorsal horn touch-evoked allodynia in the mononeuropathic rat: a Fos study. Pain 92, 389.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., Yaksh, T.L., 1994a. Quantitative assessment of tactile allodynia in the rat paw. J. Neurosci. Methods 53, 55.

Chaplan, S.R., Pogrel, J.W., Yaksh, T.L., 1994b. Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia. J. Pharmacol. Exp. Ther. 269, 1117.

Dixon, W.J., 1980. Efficient analysis of experimental observations. Annu. Rev. Pharmacol. Toxicol. 20, 441.

Hirata, H., Albillos, A., Fernandez, F., Medrano, J., Jurkiewicz, A., Garcia, A.G., 1997. Omega-conotoxins block neurotransmission in the rat vas deferens by binding to different presynaptic sites on the N-type Ca^{2+} channel. Eur. J. Pharmacol. 321, 217.

Kim, S., Chung, J., 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50, 355.

Levy, R., Leiphart, J., Dills, C., 1994. Analgesic action of acute and chronic

intraspinally administered opiate and alpha 2-adrenergic agonists in chronic neuropathic pain. Stereotact. Funct. Neurosurg. 62, 279.

Lewis, R.J., Nielsen, K.J., Craik, D.J., Loughnan, M.L., Adams, D.A., Sharpe, I.A., Luchian, T., Adams, D.J., Bond, T., Thomas, L., Jones, A., Matheson, J.L., Drinkwater, R., Andrews, P.R., Alewood, P.F., 2000. Novel ω-conotoxins from *Conus catus* discriminate among neuronal calcium channel subtypes. J. Biol. Chem. 275, 35335.

Malmberg, A.B., Yaksh, T.L., 1994. Voltage-sensitive calcium channels in spinal nociceptive processing: blockade of N- and P-type channels inhibits formalin-induced nociception. J. Neurosci. 14, 4882.

Malmberg, A.B., Yaksh, T.L., 1995. Effect of continuous intrathecal infusion of omega-conopeptides, N-type calcium-channel blockers, on behavior and antinociception in the formalin and hot-plate tests in rats. Pain 60, 83.

Nielsen, K.J., Schroeder, T., Lewis, R., 2000. Structure–activity relationships of omega-conotoxins at N-type voltage-sensitive calcium channels. J. Mol. Recognit. 13, 55.

Omote, K., Kawamata, M., Satoh, O., Iwasaki, H., Namiki, A., 1996. Spinal antinociceptive action of an N-type voltage-dependent calcium channel blocker and the synergistic interaction with morphine. Anesthesiology 84, 636.

Penn, R.D., Paice, J.A., 2000. Adverse effects associated with the intrathecal administration of ziconotide. Pain 85, 291.

Plummer, M.R., Logothetis, D.E., Hess, P., 1989. Elementary properties and pharmacological sensitivities of calcium channels in mammalian peripheral neurons. Neuron 2, 1453.

Portenoy, R.K., Foley, K.M., Inturrisi, C.E., 1990. The nature of opioid responsiveness and its implications for neuropathic pain: new hypotheses derived from studies of opioid infusions. Pain 43, 273.

Rhim, H., Miller, R.J., 1994. Opioid receptors modulate diverse types of calcium channels in the nucleus tractus solitarius of the rat. J. Neurosci. 14, 7608.

Sanger, G.J., Ellis, E.S., Harries, M.H., Tilford, N.S., Wardle, K.A., Benham, C.D., 2000. Rank-order inhibition by omega-conotoxins in human and animal autonomic nerve preparations. Eur. J. Pharmacol. 388, 89.

Suzuki, R., Chapman, V., Dickenson, A.H., 1999. The effectiveness of spinal and systemic morphine on rat dorsal horn neuronal responses in the spinal nerve ligation model of neuropathic pain. Pain 80, 215.

Vega, T., De Pascual, R., Bulbena, O., Garcia, A.G., 1995. Effects of omega-toxins on noradrenergic neurotransmission in beating guinea pig atria. Eur. J. Pharmacol. 276, 231.

Wang, Y.X., Gao, D., Pettus, M., Phillips, C., Bowersox, S.S., 2000. Interactions of intrathecally administered ziconotide, a selective blocker of neuronal N-type voltage-sensitive calcium channels, with morphine on nociception in rats. Pain 84, 271.

Wright, C.E., Robertson, A.D., Whorlow, S.L., Angus, J.A., 2000. Cardiovascular and autonomic effects of omega-conotoxins MVIIA and CVID in conscious rabbits and isolated tissue assays. Br. J. Pharmacol. 131, 1325.

Yaksh, T., Rudy, T., 1976. Chronic catheterization of the spinal subarachnoid space. Physiol. Behav. 17, 1031.