British Journal of Pharmacology (2000) 131, 1325–1336

www.nature.com/bjp

Cardiovascular and autonomic effects of ω-conotoxins MVIIA and CVID in conscious rabbits and isolated tissue assays

*,[1]Christine E. Wright, [1]Alan D. Robertson, [1]Sarah L. Whorlow & [1]James A. Angus

[1]Department of Pharmacology, The University of Melbourne, Victoria 3010, Australia

1 The effects of a novel N-type voltage-operated calcium channel antagonist, ω-conotoxin CVID, were compared with ω-conotoxin MVIIA on sympathetic-evoked activation of right atria (RA), small mesenteric arteries (MA) and vasa deferentia (VD) isolated from the rat. Their effects were also compared on blood pressure and cardiovascular reflexes in conscious rabbits.

2 The pIC_{50} values for MVIIA and CVID, respectively, for inhibiting sympathetic-evoked responses were equivalent in RA (8.7 and 8.7) and VD (9.0 and 8.7); however, in MA the values were 8.4 and 7.7. The cardiac to vascular (RA/MA) potency ratios, antilog (plog RA – plog MA), for MVIIA and CVID were 2 and 10. The offset rates for CVID and MVIIA were rapid, and peptide reapplication caused rapid onset of blockade, suggesting limited desensitization.

3 In the conscious rabbit, CVID and MVIIA (100 $\mu g\ kg^{-1}$ i.v.) caused a similar fall in blood pressure and a tachycardia that rapidly reached maximum. Both peptides decreased the vagal- and sympathetic-mediated components of the baroreflex, but had no effect on the vagal nasopharyngeal reflex. The orthostatic reflex to 90° tilt was blocked by MVIIA with sustained postural hypotension for $\geqslant$90 min after administration. In contrast, CVID caused postural hypotension at 30 min which recovered rapidly.

4 Neither CVID nor MVIIA (3 $\mu g\ kg^{-1}$ i.t.) significantly altered cardiovascular variables or autonomic reflexes.

5 In conclusion, CVID appears to be relatively weak at inhibiting the reflex response to tilt consistent with its weaker inhibition of rat mesenteric artery constriction to perivascular nerve stimulation. This may point to subtype N-type calcium channel selectivity.
British Journal of Pharmacology (2000) **131,** 1325–1336

Keywords: ω-Conotoxin MVIIA; ω-conotoxin CVID; ω-conotoxin GVIA; N-type calcium channels; sympathetic neurotransmission; baroreceptor reflex; orthostatic hypotension; nasopharyngeal reflex

Abbreviations: CVID, ω-conotoxin CVID; GVIA, ω-conotoxin GVIA; MVIIA, ω-conotoxin MVIIA

Introduction

Intrathecal infusion of a selective N-type voltage-operated calcium channel antagonist, ω-conotoxin MVIIA (SNX111 or ziconotide), is under clinical trial for the treatment of opioid-resistant neuropathic pain in patients with cancer or AIDS (Brose *et al*., 1997; Shen *et al*., 2000). A recent report indicates that some serious side effects are associated with central sites of N-channel blockade and peripheral autonomic dysfunction (Penn & Paice, 2000). Studies from our laboratory have established that the potent but slow onset and offset N-channel antagonist, ω-conotoxin GVIA, impairs the baroreceptor-heart rate reflex (Pruneau & Angus, 1990). GVIA causes postural hypotension (Hawkes *et al*., 1995) in the conscious rabbit after intravenous administration, but appears to spare the nasopharyngeal and Bezold-Jarisch like reflexes (Wright & Angus, 1995). When given intravenously, the acute sympatholytic and vagolytic effects of GVIA resolve after 48 h, but a second hypotensive and bradycardic phase lasts a further 96 h (Wright & Angus, 1997). When GVIA is given into the cerebral ventricle the haemodynamic (hypotensive) effects are blunted and have a 48 h delay in onset (Whorlow *et al*., 1994). Given the theoretical advantages of a faster onset and offset N-channel antagonist for allodynia and neuropathic pain, there is much interest in novel but still selective agents for blocking N-type voltage-operated calcium channels.

*Author for correspondence; E-mail: cewright@unimelb.edu.au

The aim of the present work was to characterize the sympatholytic potency, cardiovascular and autonomic reflex effects of a novel ω-conotoxin, CVID, from the marine cone snail, *Conus catus*. This peptide of 27 amino acids has three disulphide bridges consistent with the classical N-channel antagonists GVIA and MVIIA. However, loop 4 of CVID shows sequence divergence that may stabilize the conformation of loop 2 as determined from the solution structure by 3-dimensional ^{1}H NMR (Lewis *et al*., 2000; Nielsen *et al*., 2000). CVID has a similar potency as GVIA and MVIIA in displacing ^{125}I-GVIA from N-type calcium channels in rat brain, but was 10 fold more potent than GVIA in inhibiting the current under voltage clamp through the cloned $\alpha_{1B\text{-}D}$, central N-type calcium channel expressed in *Xenopus* oocytes (Lewis *et al*., 2000). In this study, MVIIA has been compared with CVID in three isolated sympathetic nerve assays to assess, with precision, relative potencies. In the conscious rabbit experiments, both peptides were compared by the intravenous and intrathecal routes and show similar onset and offset characteristics. Earlier work showed that a significant part of the hypotension to MVIIA in conscious rats was due to histamine release from mast cell degranulation. This effect was blunted by histamine antagonists or by substituting alanine for arginine at amino acid position 10 (Bowersox *et al*., 1992). The present work tested whether histamine release was involved in the effects of MVIIA.

Methods

The study was approved by the University of Melbourne animal ethics and experimentation committee in accordance with the guidelines of the National Health and Medical Research Council of Australia.

In vitro studies

Sprague-Dawley rats (250–300 g) were killed by exposure to 80% CO_2 in O_2 and exsanguination. Right atria and mesentery were bathed during dissection, mounting and the experiment in a physiological salt solution (PSS) of the following composition (in mM): NaCl 119, KCl 4.7, KH_2PO_4 1.18, $MgSO_4$ 1.17, $NaHCO_3$ 25, $CaCl_2$ 2.5, ethylenediaminetetracetic acid (EDTA) 0.026, glucose 11 and saturated with 95% O_2 and 5% CO_2. Vasa deferentia were bathed in Mg^{2+}-free PSS.

Rat isolated small mesenteric artery

Small arteries (300–400 μm diam.) were dissected from the mesenteric bed and mounted as ring preparations in a dual chamber isometric myograph (J.P. Trading, Aarhus, Denmark). Two vessels were set up in a 15 ml bath (at 37°C) and stretched to an internal circumference equivalent to 90% of the internal diameter of the vessel if it had been relaxed and under a transmural pressure of 100 mmHg (Mulvany & Halpern, 1977). Platinum electrodes (5 μm thick) were contained in the mounting supports of the myograph to deliver square wave field stimulation using Grass SD9/S88 stimulators. Output from the transducer amplifier was recorded on a flat bed recorder (Model 320, W & W Scientific Instruments, Basle, Switzerland).

Vessels were allowed to equilibrate for 30 min following the normalization procedure and were then maximally activated with high potassium PSS (standard PSS with an equimolar exchange of KCl for NaCl, i.e. K^+ 124 mM termed KPSS) followed by 10 μM noradrenaline. Pre-junctional α_2-adrenoceptors were irreversibly blocked using a receptor protection procedure (Angus *et al*., 1988). Vessels were first exposed to prazosin (0.1 μM) for 5 min and then benextramine (3 μM) for a further 5 min. Arteries were washed thoroughly over a period of 30 min. Electrical field stimulation was applied at 30 V (dial setting), 0.25 ms duration, 24 Hz frequency for a 3 s train every min. This stimulus train was chosen on the basis of data from Angus *et al*. (1988) where such stimulation caused contractions of 40–60% of that to KPSS. Tetrodotoxin (TTX, 0.1 μM, 1 min incubation) was added to confirm the neural mediation of responses to electrical stimulation; voltage was adjusted until contractile responses were totally abolished in the presence of TTX. Vessels were washed thoroughly to remove TTX from the bath. Two trains of control field stimulation were applied followed by noradrenaline 10 μM. The effects of vehicle (H_2O), MVIIA ($10^{-9.28}$–$10^{-6.28}$ M) or CVID ($10^{-9.14}$–$10^{-6.14}$ M) were measured as the per cent inhibition of the second set of control field stimulation. Each drug concentration was added cumulatively and equilibrated for 30 min before the responses to electrical field stimulation were reassessed.

Rat isolated right atrium

The right atria were isolated from rat hearts and placed vertically on stainless steel S-shaped hooks attached to Grass FT03C force transducers (Grass Instruments, Quincy, MA, U.S.A.) in PSS-filled 10 ml glass-jacketed organ baths heated to 37°C. Each partially stretched atrium rested against two punctate platinum electrodes protruding from the tissue holder 3 mm apart that recorded the spontaneous surface electrogram (monitored on a dual beam 10 MHz storage oscilloscope, Model T912, Tektronix, Guernsey, U.K.). This signal was amplified (Baker Medical Research Institute (BMRI) amplifier Model 108, Melbourne, Australia) and used to trigger a rate meter (BMRI Model 173). Atrial period and force of contraction were continuously recorded on a chart recorder (Neotrace 600ZF, Neomedix, Sydney, Australia). To examine the sympathetic response in the absence of bradycardia, the atria were equilibrated for 1 h in the presence of atropine (1 μM). Three control sets of four field pulse stimuli (100 V, 2 ms duration, 2 Hz) were delivered *via* a Grass S88C dual stimulator and a pair of platinum wire field electrodes that were arranged parallel to the atrium. The control stimuli were followed by addition of vehicle (H_2O), MVIIA ($10^{-10.28}$–$10^{-7.28}$ M) or CVID ($10^{-10.14}$–$10^{-7.14}$ M). Each drug concentration was added cumulatively and equilibrated for 30 min before the responses to field stimulation were reassessed. In separate tissues, the effects of MVIIA ($10^{-10.28}$–$10^{-7.28}$ M) were tested in the absence or presence of histamine H_1 receptor block with mepyramine (10^{-7} M) and H_2 and H_3 receptor block with burimamide (10^{-4} M) (Ishikawa & Sperelakis, 1987; Hey *et al*., 1992).

Rat isolated vas deferens

Rat vasa deferentia were dissected with capsular connective tissue intact and set up in 5 ml organ baths at 37°C in Mg^{2+}-free PSS. The upper (epididymal) end was attached to an isometric force transducer (Grass FTO3C) and the lower (prostatic) end tied to a fixed support between two parallel platinum field electrodes (5 mm apart, 5 mm long). The tissue was initially stretched by 2 g force and allowed to equilibrate for 20 min. The vas deferens was continuously stimulated (Grass S88 stimulator) to contract (twitch) with a single electrical field pulse (150 V, 0.5 ms duration) delivered every 20 s. Output from the transducer amplifier was recorded on a flat bed recorder (Linearcorder WR3300, Graphtec, Tokyo, Japan). The effects of vehicle (H_2O), MVIIA ($10^{-10.28}$–$10^{-7.28}$ M) or CVID ($10^{-10.14}$–$10^{-7.14}$ M) were measured as the per cent inhibition of the pre-drug twitch force. Each drug concentration was added cumulatively and equilibrated for 20 min before the effects on the twitch response were assessed.

An additional set of experiments was designed to test whether these peptides caused desensitization with prolonged incubation. Rat isolated vasa deferentia were set up as above. Vehicle (H_2O; paired with each peptide experiment), MVIIA (10 nM) or CVID (30 nM) was added and incubated for 240 min with twitch responses to electrical stimulation tested every 15–30 min. Peptide or vehicle was washed out at 240 min. Thirty minutes later (at 270 min), MVIIA (10 nM) or CVID (30 nM) was applied to both the paired vehicle- or respective peptide-treated tissues and twitch responses re-tested at 285 and 300 min.

In vivo studies

Experimental preparation

On the day of each experiment, the central ear artery and marginal ear vein were cannulated under local anaesthesia (0.5% lignocaine hydrochloride; Xylocaine, Astra, Sydney,

Australia). For rabbits with implanted intrathecal catheters (see below), the catheter was retrieved from the back of the neck under local anaesthesia. The ear artery catheter was connected to a Cobe CDX pressure transducer (Cobe, Lakewood, CO, U.S.A.) for the measurement of phasic and mean arterial blood pressure (MAP). The phasic blood pressure signal triggered a rate meter (Model 173, BMRI, Melbourne, Australia) for the measurement of heart rate (HR). MAP and HR were monitored on a Grass polygraph (Model 7D). The ear vein catheter was for administration of drugs. Rabbits rested quietly in a polycarbonate restrainer (Nalgene, Nalge, Rochester, NY, U.S.A.) for ~45 min before experiments commenced.

Intravenous peptide administration

Protocol The effects of i.v. administration of vehicle or peptides were assessed on MAP, HR, the baroreflex and nasopharyngeal reflex in conscious rabbits (2.49 ± 0.09 kg; $n=8$). Rabbits were randomly allocated to treatment groups and 5–7 days allowed between each of the three treatments. The nasopharyngeal reflex was induced by passing about 20 ml cigarette smoke through a plastic tube connected to a 20 ml syringe near the nostrils of the rabbit over 10–12 s. When MAP and HR had returned to baseline, the baroreceptor-heart rate reflex (baroreflex) was assessed using the steady-state method (Head & McCarty, 1987; Head & Adams, 1992). Alternate stepwise increases and decreases in MAP were evoked by i.v. injections of phenylephrine (1–150 μl of 250 μg ml^{-1}) and sodium nitroprusside (5–500 μl of 1 mg ml^{-1}), respectively, to achieve changes in MAP of ± 5–35 mmHg from baseline. Doses were chosen randomly, however, an increase in MAP was always followed by a decrease in MAP to prevent a shift in resting parameters. Vehicle (saline; 0.9% NaCl), MVIIA (100 μg kg^{-1}) or CVID (100 μg kg^{-1} was then administered as an i.v. bolus and MAP and HR monitored for 30 min. The nasopharyngeal reflex and baroreflex were then retested and rabbits returned to their home cages between experimental days.

In an additional group of rabbits (3.0 ± 0.4 kg, $n=4$), the effects of vehicle or peptide treatment on orthostatic responses to 90° head-up tilt were assessed. The rabbit box was padded with foam to alleviate the stress of head-up tilting. The orthostatic response to head-up tilt was assessed by rotating the box through 90° in less than 1 s, holding the box in this position for 1 min, then back to the horizontal position (Hawkes *et al*., 1995; Wright & Angus, 1997). Three to four trial tilts were performed to accustom each rabbit to the procedure; three control tilts were then recorded before the rabbit received either vehicle (saline), MVIIA (100 μg kg^{-1}) or CVID (100 μg kg^{-1}) as an i.v. bolus. Head-up tilts were repeated 30, 60 and 90 min post-peptide or vehicle administration. Rabbits returned to their home cages between experimental days. Rabbits were randomly allocated to treatment groups and 5 days allowed between each of the three treatments.

To examine whether the hypotension induced by MVIIA in the conscious rabbit was perhaps in part mediated by release of histamine, experiments were performed in four additional animals (2.45 ± 0.09 kg) in the presence of mepyramine (0.8 mg kg^{-1} i.v. bolus) and cimetidine (5 mg kg^{-1} i.v. bolus). Each rabbit received either vehicle or MVIIA in a randomized order. Five minutes after administration of the histamine antagonists, vehicle (saline) or MVIIA (100 μg kg^{-1}) was given as an i.v. bolus and MAP and HR monitored for 90 min.

Intrathecal peptide administration

Surgical implantation of intrathecal catheter Male and female New Zealand white rabbits (2.09 ± 0.06 kg; $n=18$) were anaesthetized with i.v. Saffan (alphaxalone and alphadolone, 6 and 2 mg kg^{-1}, respectively; Pitman-Moore, Sydney, Australia), intubated and surgical anaesthesia maintained with halothane (Rhône Mérieux, Melbourne, Australia). A small sterile saline-filled polyethylene catheter (SP8, Dural Plastics, Sydney, Australia; i.d. 0.20 mm; dead space ~20 μl) was inserted into the subarachnoidal space with the tip at ~T1 according to a modification of the method described by Yaksh & Rudy (1976). Briefly, each rabbit was placed in a stereotaxic frame with its head flexed forward. A midline incision was made at the back of the neck and the muscles parted to expose the atlanto-occipital membrane. A small hole was made in the caudal part of the membrane with a 21-gauge needle and the catheter inserted 7 cm caudal. The catheter was sutured to the membrane with a purse-string suture, and a further anchor point was made in the muscle. The external end of the catheter was tunnelled subcutaneously to the back of the neck. Rabbits were administered the analgesic buprenorphine (0.5 mg kg^{-1} s.c.; Temgesic, Reckitt & Colman, Sydney, Australia), and the antibiotic Tribrissen (trimethorprim and sulphadiazine, 5 and 25 mg kg^{-1} i.v.; Jurox, Sydney, Australia) at the completion of surgery. Experiments commenced 5–7 days following surgery.

Protocol The effect of i.t. MVIIA, CVID or vehicle on MAP, HR, the baroreflex, nasopharyngeal reflex and the orthostatic response to head-up tilt were examined. The nasopharyngeal reflex, baroreflex curves and tilt responses were assessed as above. Rabbits were then administered either vehicle (50 μl sterile saline i.t. over 1 min), MVIIA or CVID (both at 3 μg kg^{-1} in 10 μl followed by 40 μl sterile saline flush). This dose was chosen on the basis of pilot experiments in three rabbits (data not shown) in which changes in cardiovascular parameters were observed, but no behavioural changes were apparent. MAP and HR were measured for 30 min and then reflex measurements repeated. To examine the pressor component of the nasopharyngeal reflex in the absence of the bradycardia, methscopolamine (50 μg kg^{-1} i.v.), a muscarinic antagonist which does not cross the blood brain barrier and affect central muscarinic receptors, was administered and 5 min later the reflex was retested. In the rabbit, this dose of methscopolamine will completely block reflex bradycardia (Wright & Angus, 1983). At 24 and 48 h post-treatment all reflexes were retested, however no further peptide or vehicle was administered. At the completion of the experiment at 48 h, rabbits were given an overdose of i.v. pentobarbitone (Lethabarb, Virbac, Sydney, Australia) and placement of the catheter was verified by i.t. injection of 50 μl Evan's Blue dye. Correct placement was determined visually with the tip of the catheter at ~T1 and spread of dye 2–4 cm rostral and caudal to the catheter tip. If the catheter was incorrectly placed, data from that animal were discarded.

Drugs

Drugs used and their sources were: burimamide (gift from James Black Foundation, Dulwich, U.K.), cimetidine (Sigma, St Louis, MO, U.S.A.), mepyramine maleate (Tocris Cookson, Bristol, U.K.), methscopolamine bromide (Upjohn,

Rydalmere, Australia), phenylephrine hydrochloride (Sigma) and sodium nitroprusside (David Bull Laboratories, Melbourne, Australia). ω-Conotoxins MVIIA and CVID were synthesized by the Centre for Drug Design and Development, The University of Queensland (St Lucia, Qld, Australia) (Lewis *et al*., 2000; Nielsen *et al*., 2000). MVIIA and CVID were dissolved in 0.9% saline (sterile saline for i.t. administration) and stored in aliquots (1 mg ml^{-1}) at −20°C until required. All other drugs were freshly dissolved in saline except burimamide which was dissolved in 0.1 M HCl then diluted in water and cimetidine which was dissolved in 5% dextrose solution and 5% propylene glycol.

Analyses and statistical methods

Data are presented as mean ± standard error of the mean (s.e.mean) of n experiments. Responses elicited by electrical field stimulation of vasa deferentia are expressed as a percentage of the baseline twitch contraction and of small mesenteric arteries as a percentage of the second control contraction. Sympathetic responses to electrical field stimulation of right atria are presented as a percentage of the control response to the third set of four field pulses. For the rabbit experiment, average MAP and HR values presented in the text have been rounded to the nearest whole number. The average s.e.mean within tissues (or rabbits) was calculated from repeated measures analysis of variance (ANOVA) using the pooled estimate of error from the residual mean square as (error mean square/number of tissues, or rabbits)$^{0.5}$ after sums of squares between tissues (or rabbits) and between peptide concentrations (or times) had been subtracted from the total sums of squares for each treatment group (Snedecor & Cochran, 1989). These average s.e.means are located on the lines shown in Figures 1, 4 and 5 (Wright *et al*., 1987; Wright & Angus, 1996). For rat atrium, mesenteric artery and vas deferens experiments, sympathetic responses were compared within and between groups by repeated measures ANOVA with Greenhouse-Geisser correction for correlation (Ludbrook, 1994), calculated by means of the statistical program SuperANOVA™ 1.11 for Macintosh. pIC_{50} values for MVIIA and CVID were compared within tissue type by unpaired Student's t-test. Orthostatic responses to 90° head-up tilt were measured at 5 s (peak cardiovascular response) and 50 s into each 1 min tilt. Responses to phenylephrine, sodium nitroprusside and cigarette smoke were measured as peak changes in MAP and HR from baseline. The baroreceptor-heart rate reflex (baroreflex) curves were analysed by fitting the MAP and corresponding HR changes to a sigmoidal logistic equation characterized by (i) HR range (beats min^{-1}) between upper and lower plateaus of the curve; (ii) median blood pressure (MAP_{50}; mmHg); (iii) average gain (beats min^{-1} $mmHg^{-1}$) over the linear portion of the curve; and (iv) lower HR plateau (beats min^{-1}) (Head & McCarty, 1987).

In the intravenous study, comparisons of baroreflex parameters were compared within the group before and after treatment by Student's t-test for paired data. Values for MAP or HR were compared before and 5 min after histamine antagonist administration by Student's t-test for paired data. Values for baseline MAP or HR at 0 min were compared between groups by 1-way ANOVA. Baroreflex parameters and nasopharyngeal reflex responses before and after treatment, and MAP and HR values measured for 30 min following treatment, were compared within and between groups by repeated measures ANOVA with Greenhouse-Geisser correction for correlation. This means was also used to compare orthostatic responses to tilt at 0, 30, 60 and 90 min post-treatment. In the intrathecal study, comparisons of MAP, HR, orthostatic responses to tilt, nasopharyngeal and baroreflex parameters were made within and between groups by repeated measures ANOVA with Greenhouse-Geisser correction for correlation. MAP or HR values at 0 min were compared between groups by 1-way ANOVA.

Probability values less than 0.05 were accepted as statistically significant.

Results

In vitro studies

Rat isolated mesenteric artery

The average internal diameter of the mesenteric arteries at L_{100} was 363 ± 9 μm (n = 16). The contractions in response to electrical field stimulation were abolished in the presence of TTX (0.1 μM) indicating mediation by intramural nerves. Responses to the second set of control field stimulation (C2) were similar in all drug treatment groups. Contractile responses were unaffected by vehicle administration (n = 4) and were consistent throughout the experiment. MVIIA and CVID inhibited the contraction to nerve stimulation in a concentration-dependent manner (Figure 1, top panel) with pIC_{50} values of 8.4 ± 0.11 (n = 6) and 7.7 ± 0.04 (n = 6), respectively (Table 1). MVIIA was significantly more potent than CVID (P < 0.01, 1-way ANOVA).

Rat isolated right atria

In isolated right atria in the presence of atropine (1 μM), tachycardic responses were unaffected by vehicle (P = 0.17, repeated measures ANOVA; Figure 1, middle panel). MVIIA and CVID completely inhibited the sympathetic response by 100 nM, with similar pIC_{50} values of 8.7 ± 0.20 and 8.7 ± 0.23, respectively (n = 4 each; Table 1). In separate tissues, the effects of MVIIA were tested in the presence or absence of the histamine H_1 and H_2 receptor antagonists, mepyramine (10^{-7} M) and burimamide (10^{-4} M). MVIIA inhibited atrial responses with comparable pIC_{50} values of 8.9 ± 0.13 (n = 4) in the presence and 8.6 ± 0.03 (n = 4) in the absence of histamine receptor antagonism (P = 0.09, unpaired t-test; data not shown).

Rat isolated vasa deferentia

In vasa deferentia stimulated with single pulses every 20 s, there was a gradual decrease in the twitch response to nerve stimulation in the vehicle treatment group over time (P = 0.013, repeated measures ANOVA). MVIIA and CVID completely inhibited the twitch response by 100 nM (Figure 1, bottom panel). MVIIA was significantly more potent (pIC_{50} 9.0 ± 0.07, n = 7) than CVID (pIC_{50} 8.7 ± 0.07, n = 8; P < 0.005, unpaired t-test; Table 1).

A comparison of pIC_{50} values shows that, generally speaking, all three ω-conotoxins were equipotent at inhibiting sympathetic nerve-mediated responses in the vas deferens and right atrium assays with mean pIC_{50} values of 8.7−9. In contrast, CVID appears to be significantly less potent in the mesenteric artery assay than MVIIA and GVIA. A correlation of the pIC_{50} values for pairs of comparisons show the outlier for CVID (Figure 2). Another way to

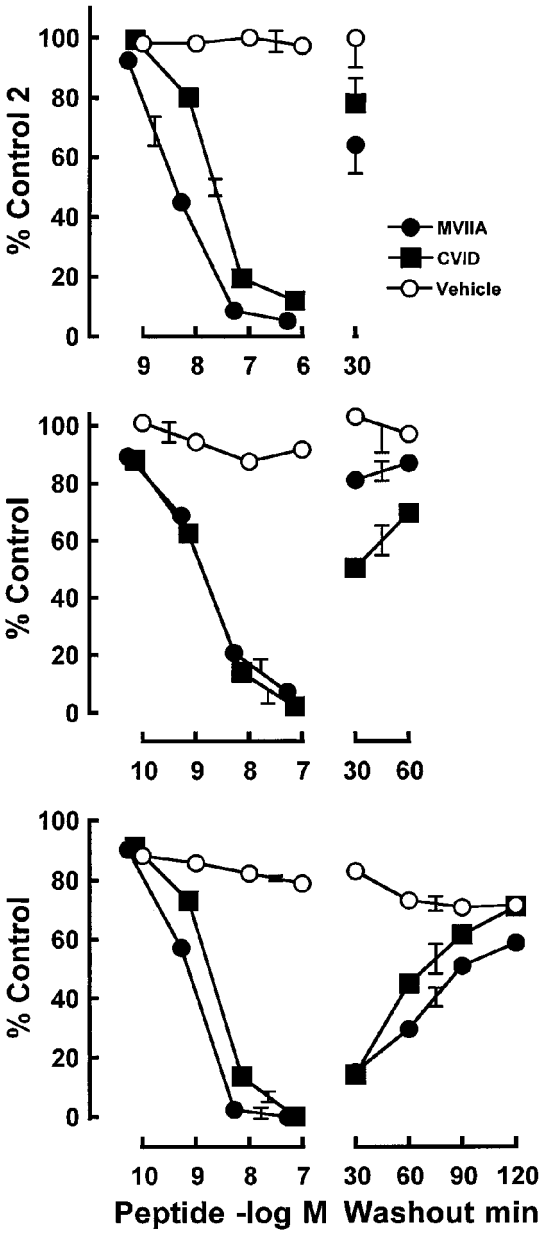

Figure 1 Upper panel: Effects of vehicle (H_2O; $n=4$), ω-conotoxin MVIIA ($n=6$) or ω-conotoxin CVID ($n=6$) on contractions elicited by electrical field stimulation of rat isolated small mesenteric artery. The recovery of responses following 30 min washout of vehicle or peptide is also shown. Responses are expressed as a percentage of the contraction to the second control stimulation (C2). Error bars on the lines are average s.e.mean from ANOVA (see Methods), and on washout values are ±1 s.e.mean. Middle panel: Effects of vehicle (H_2O; $n=4$), ω-conotoxin MVIIA ($n=4$) or ω-conotoxin CVID ($n=4$) on tachycardic responses to four field pulses of rat isolated right atria in the presence of 1 μM atropine. The recovery of responses following 30 and 60 min washout of vehicle or peptide is also shown. Responses are expressed as a percentage of the control tachycardia. Error bars on the lines are average s.e.mean from ANOVA. Lower panel: Effects of vehicle (H_2O; $n=5$), ω-conotoxin MVIIA ($n=7$) or ω-conotoxin CVID ($n=8$) on contractions elicited by electrical field stimulation of rat isolated vasa deferentia. The recovery of responses following 30 to 120 min washout of vehicle or peptide is also shown. Responses are expressed as a percentage of the control twitch contraction. Error bars on the lines are average s.e.mean from ANOVA.

express this is to consider a cardiac to vascular sympatholytic potency ratio from antilog (pIC_{50} right atrium − pIC_{50} mesenteric artery. This gives a 10 fold selectivity for CVID ($P=0.0007$, unpaired t-test), but only 2 or less for MVIIA and GVIA (Table 1).

Figure 3 shows the effects of prolonged incubation (4 h) with either MVIIA (10 nM; $n=5$) or CVID (30 nM; $n=5$) on the rat vas deferens twitch to nerve stimulation. The degree of inhibition (80–90% by 30 min of incubation) caused by each peptide was similar and stable over the 4 h period ($P>0.05$, repeated measures ANOVA). There was significant fade in the twitch response over time in each set of paired vehicle-treated tissues ($P<0.0005$, repeated measures ANOVA); by 4 h twitch responses were only 46% of baseline ($n=5$ each; Figure 3). Upon washout, responses in the peptide-treated tissues recovered to levels similar to those of the vehicle groups. Re-administration of MVIIA (10 nM) or CVID (30 nM), as well as to their respective paired vehicle-treated tissues, at 270 min caused rapid inhibition (88–100%) of responses in all groups (Figure 3). There was no evidence of desensitization to either peptide.

In vivo studies

Effect of treatments on MAP and HR

Intravenous peptide administration Baseline (0 min) values for MAP and HR were comparable in each treatment group ($P>0.05$, 1-way ANOVA). Following i.v. administration of vehicle (saline; $n=6$), there was no significant change in MAP or HR from the 0 min (just prior to administration) baseline values of 74±1 mmHg and 189±3 beats min^{-1}, respectively ($P>0.05$, repeated measures ANOVA; Figure 4). The i.v. administration of 100 μg kg^{-1} MVIIA ($n=6$) or CVID ($n=6$) caused hypotension and tachycardia in conscious rabbits (Figure 4). In the MVIIA group the change in MAP appeared to be biphasic with a fall from the 0 min baseline of 67±2 mmHg to 56±2 mmHg after 5 min, then an increase to 59±2 mmHg by 9 min before a decrease to 55±2 mmHg by 15 min (Figure 4). There was a tachycardia of 60±6 beats min^{-1} from the 0 min baseline of 187±12 beats min^{-1} by 5 min after MVIIA administration (Figure 4).

The administration of CVID caused a peak fall in MAP of 14±3 mmHg from the 0 min baseline of 73±3 mmHg after 15 min; HR increased by 62±5 beats min^{-1} from 183±7 beats min^{-1} (Figure 4). The effects of MVIIA and CVID on MAP were similar over 30 min ($P>0.05$, repeated measures ANOVA). The tachycardia following MVIIA administration was significantly greater than that after CVID ($P=0.027$; repeated measures ANOVA).

In a separate rabbit group, mepyramine and cimetidine were administered 5 min prior to i.v. vehicle ($n=4$) or MVIIA (100 μg kg^{-1}; $n=4$). These histamine antagonists did not cause a change in HR or MAP ($P>0.05$, paired $t=$test) during this 5 min period in either group. Over the 90 min treatment period in the vehicle group, there was no significant change in HR ($P=0.54$) or MAP ($P=0.70$, repeated measures ANOVA) from the 0 min baseline values of 200±12 beats min^{-1} and 74±5 mmHg, respectively (data not shown). The hypotension ($P=0.305$) and tachycardia ($P=0.160$, repeated measures ANOVA) observed following i.v. MVIIA (data not shown) were similar in the presence of the histamine antagonists to the effects of the peptide in the absence described above.

Intrathecal peptide administration Baseline (0 min) values for MAP and HR were comparable in each treatment group

Table 1 Potency (pIC_{50}) of ω-conotoxins MVIIA, CVID and GVIA in rat isolated small mesenteric artery, right atrium and vas deferens

ω-Conotoxin	*Mesenteric artery* pIC_{50}	n	*Right atrium* pIC_{50}	n	*Vas deferens* pIC_{50}	n	*Cardiac/vascular selectivity ratio*[a]
MVIIA	8.4±0.11	6	8.7±0.20	4	9.0±0.07	7	2
CVID	7.7±0.04*	6	8.7±0.23	4	8.7±0.07*	8	10
GVIA[b]	8.8±0.06	6	9.0±0.09	6	8.9±0.05	5	1.6

Data are presented as mean±s.e.mean. pIC_{50} values correspond to the negative log concentration of drug required to cause 50% of maximal inhibition of response. *n*, number of tissues. [a]Cardiac/vascular selectivity ratio is antilog(pIC_{50} right atrium − pIC_{50} mesenteric artery). *$P<0.05$ ω-conotoxin MVIIA compared with ω-conotoxin CVID in same tissue type, unpaired *t*-test. [b]Previously published data from our laboratory for comparison (Lew *et al*., 1997).

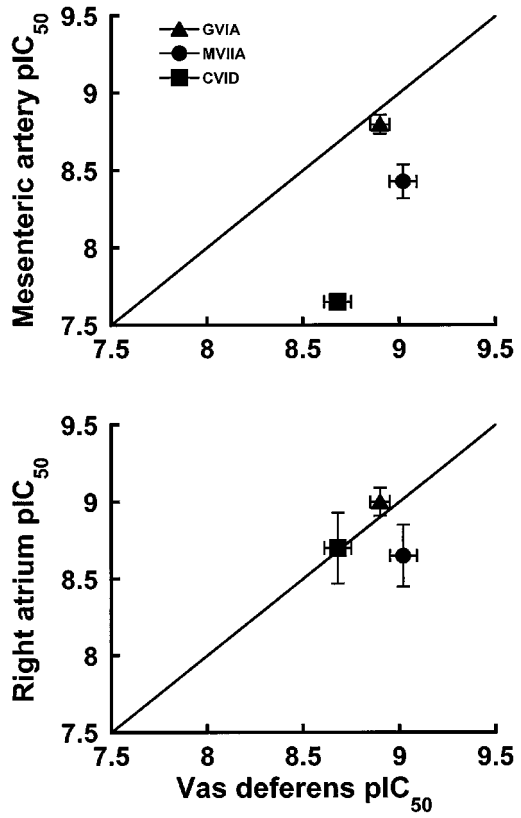

Figure 2 Correlation graphs of pIC_{50} values for twin tissue comparisons of ω-conotoxins GVIA, MVIIA and CVID in rat isolated mesenteric artery and vas deferens (top panel) and rat right atrium and vas deferens (bottom panel). Points are mean±s.e.mean pIC_{50} values as shown in Table 1. GVIA values are reproduced from previously published data from our laboratory for comparison (Lew *et al*., 1997).

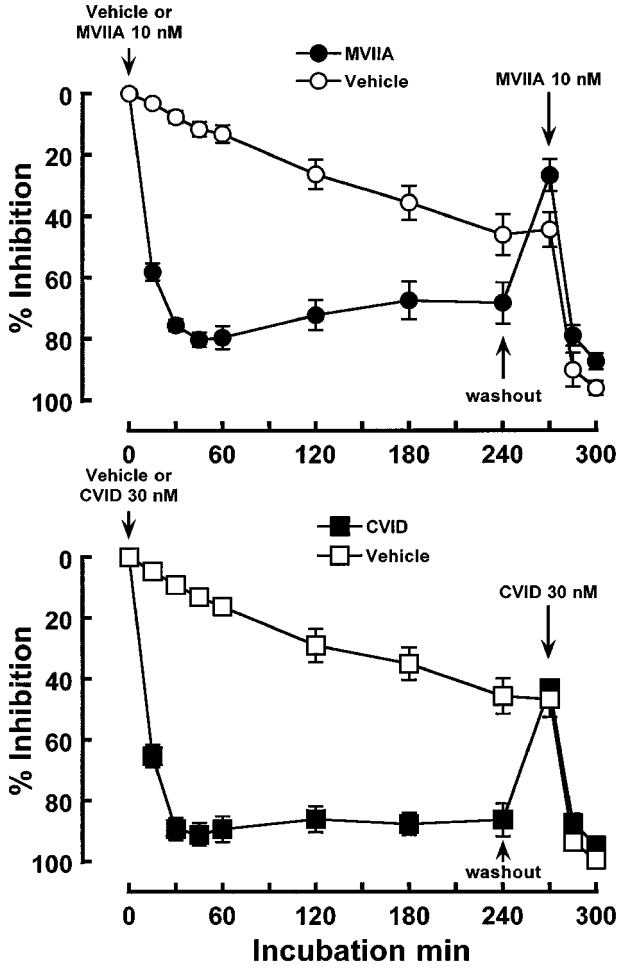

Figure 3 Effects of 240 min incubation with vehicle (H_2O; $n=5$ each), ω-conotoxin MVIIA (10 nM; MVIIA, $n=5$; upper panel) or ω-conotoxin CVID (30 nM; CVID, $n=5$; lower panel) on contractions elicited by electrical field stimulation of rat isolated vasa deferentia. After 240 min, vehicle or peptide was washed out; at 270 min, MVIIA 10 nM (upper panel) or CVID 30 nM (lower panel) was applied to both the vehicle- and peptide-treated tissues. Responses are expressed as a percentage inhibition of the control twitch contraction. Error bars are ±1 s.e.mean.

($P>0.05$, 1-way ANOVA). In the 30 min following i.t. administration of vehicle (sterile saline; $n=4$), 3 μg kg^{-1} MVIIA ($n=4$) or 3 μg kg^{-1} CVID ($n=5$) there were no significant changes in MAP ($P=0.405$) or HR ($P=0.110$, repeated measures ANOVA; Figure 5). Average MAP and HR were also similar within and between treatment groups over the 48 h experimental period ($P=0.364$ and $P=0.794$, respectively, repeated measures ANOVA; Table 3).

Behavioural observations and i.t. catheter placement The intrathecal dose of 3 μg kg^{-1} MVIIA or CVID appeared to be on the threshold for behavioural effects. In the MVIIA group, two out of five animals exhibited behavioural changes: (i) one animal had no changes at 0–2 h, but had mild tremors if disturbed at 24 h (these were absent at 48 h); and (ii) one animal had severe tremors with nystagmus and bradycardia (−70 beats min^{-1}) within 25 min of injection; at 24 h tremors were only mild with no nystagmus and at 48 h no tremors were observed (data from this animal were not included in the group). Only one animal out of five in the CVID group showed behavioural effects with mild tremors at 2 h post-administration (at 24–48 h no further tremors were noted).

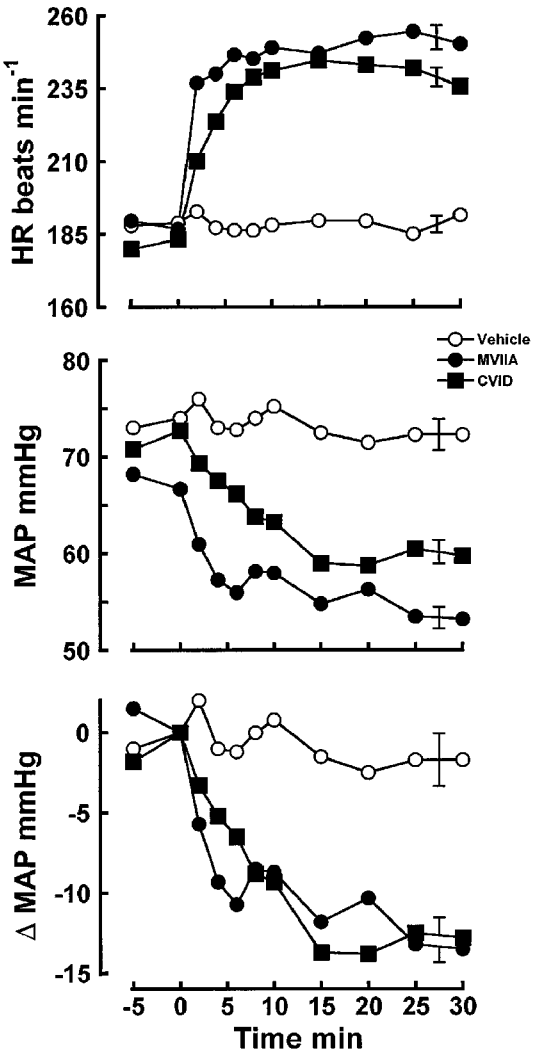

Figure 4 Heart rate (HR), mean arterial pressure (MAP) and change in MAP from 0 min baseline value (Δ MAP) from 5 min before (−5 min) to 30 min following intravenous administration of vehicle (0.9% saline; $n=6$), ω-conotoxin MVIIA 100 μg kg^{-1} ($n=6$) or ω-conotoxin CVID 100 μg kg^{-1} ($n=6$) in conscious rabbits. Treatments were administered at 0 min. Error bars are average s.e.mean from ANOVA (see Methods).

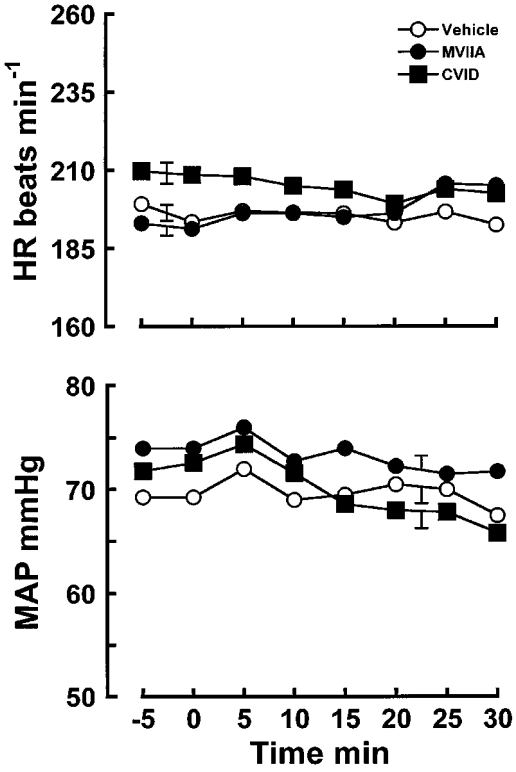

Figure 5 Heart rate (HR) and mean arterial pressure (MAP) from 5 min before (−5 min) to 30 min following intrathecal administration of vehicle (0.9% saline; $n=4$), ω-conotoxin MVIIA 3 μg kg^{-1} ($n=4$) or ω-conotoxin CVID 3 μg kg^{-1} ($n=5$) in conscious rabbits. Treatments were administered at 0 min. Error bars are average s.e.mean from ANOVA (see Methods).

Catheter placement was confirmed at autopsy following completion of the 48 h experiment in all animals. Correct placement was determined by the catheter tip at the level of T1 and a spread of injected Evan's Blue dye several cm proximal and distal from the catheter tip. In the vehicle group ($n=5$), one catheter was not successfully placed and data from that animal were discarded. Catheters in the MVIIA ($n=5$) and CVID ($n=5$) groups were all correctly placed.

Baroreceptor-heart reflex

Intravenous peptide administration Baroreflex curve parameters obtained before and after administration of vehicle, MVIIA and CVID are shown in Table 2, and the curves post-treatment are presented in Figure 6. The control (before vehicle or peptide administration) curves were not significantly different between any of the treatment groups ($P>0.05$, repeated measures ANOVA; Table 2). Following i.v. vehicle there was a small, but significant, change in the lower HR plateau of 157±8 to 149±6 beats min^{-1} ($P=0.046$, paired t-test; $n=6$); all other curve parameters were similar after vehicle administration (Table 2, Figure 6).

The i.v. administration of 100 μg kg^{-1} MVIIA ($n=6$) or CVID ($n=6$) resulted in a decrease in the average MAP ($P=0.011$) and increase in the average HR ($P=0.0001$, repeated measures ANOVA) of the baroreflex curves, which corresponds to the hypotension and tachycardia outlined above (Table 2, Figure 6). Average MAP and HR values were similar in the two peptide groups. There was also an increase in the lower HR plateau ($P=0.001$), attenuation of the HR range ($P=0.0001$) and decrease in the average gain ($P=0.0001$) of the baroreflex curves in the peptide treatment groups in comparison with their control curves and vehicle (repeated measures ANOVA; Table 2, Figure 6). Following CVID, there was a decrease in the MAP_{50} of 66±2 to 61±2 mmHg ($P=0.020$, paired t-test), however MAP_{50} values were comparable in all groups, including vehicle, pre- and post-treatment ($P=0.559$, repeated measures ANOVA; Table 2). Average gain was markedly affected by peptide administration ($P=0.0001$), with values of only −1.9±0.2 beats min^{-1} mmHg^{-1} in the MVIIA group for example (Table 2; Figure 6).

Intrathecal peptide administration Baroreflex curve parameters obtained 0–48 h after administration of vehicle, MVIIA and CVID are shown in Table 3 and the barocurves in Figure 7. The control (before treatment) curves were not significantly different between any of the treatment groups ($P>0.05$, repeated measures ANOVA; Table 3). Following i.t. administration of vehicle, MVIIA or CVID there were no significant changes in barocurve parameters within or

Table 2 Effect of intravenous vehicle, ω-conotoxin MVIIA or CVID on baroreflex curve parameters in conscious rabbits

	Vehicle n=6		*MVIIA* 100 μg kg^{-1} n=6		*CVID* 100 μg kg^{-1} n=6	
Parameter	*Pre*	*Post*	*Pre*	*Post*	*Pre*	*Post*
Low HR plateau (beats min^{-1})	157±8	149±6*	146±4	202±13*	140±5	183±6*
HR range (beats min^{-1})	189±18	206±15	196±12	78±18*	196±16	117±18*
MAP_{50} (mmHg)	68±1	67±2	62±2	61±1	66±2	61±2*
Average HR (beats min^{-1})	198±1	195±3	195±11	243±8*	183±7	226±8*
Average MAP (mmHg)	75±1	74±1	68±1	60±1*	73±2	66±2*
Average gain (beats min^{-1} mmHg^{-1})	−8.2±0.6	−9.2±1.1	−7.6±0.6	−1.9±0.2*	−9.1±1.0	−3.1±0.3*

Values are mean±s.e.mean. MAP, mean arterial pressure. HR, heart rate. MAP_{50}, MAP at half the HR range. *n*, number of animals. Average baroreceptor-heart rate reflex curve parameters obtained in conscious rabbits before (Pre) and 30–60 min after (Post) intravenous administration of vehicle (0.9% saline), ω-conotoxin MVIIA (MVIIA) or ω-conotoxin CVID (CVID). *$P<0.05$ compared with respective Pre value, Student's *t*-test for paired data.

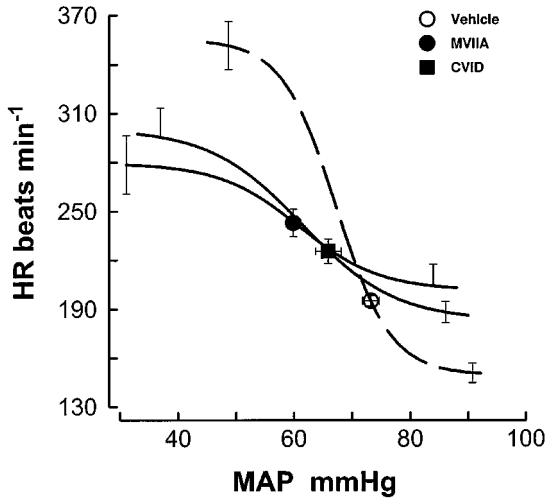

Figure 6 Average baroreceptor-heart rate reflex curves relating heart rate (HR) to mean arterial pressure (MAP) in conscious rabbits. Curves were constructed 30 min after intravenous administration of vehicle (saline; dashed line; $n=6$), ω-conotoxin MVIIA 100 μg kg^{-1} ($n=6$) or ω-conotoxin CVID 100 μg kg^{-1} ($n=6$). The symbol on the curve represents the average resting values for HR and MAP. Error bars on the symbols are s.e.mean (those not visible are contained within the symbol) and those on the curves represent the s.e.mean of the lower HR plateau (right) and the HR range (left).

between groups ($P>0.05$, repeated measures ANOVA; Table 3, Figure 7).

Nasopharyngeal reflex

Intravenous peptide administration Cigarette smoke passed near the nostrils of the rabbits resulted in profound bradycardia and a pressor response (data not shown). The latter was not analysed due to the confounding influence of the concomitant large bradycardia. The pre- (control) and post-treatment (Figure 8) bradycardic responses to smoke were not different within or between groups ($P=0.152$; repeated measures ANOVA).

Intrathecal peptide administration The nasopharyngeal reflex was assessed in the absence (at 0 min, 30 min, 24 h and 48 h) and presence (end of 30 min, 24 and 48 h time point experiments) of muscarinic receptor block with methscopolamine (50 μg kg^{-1} i.v.). In the absence of methscopolamine, bradycardic responses to smoke were comparable 0–48 h post-vehicle or peptide administration within and between groups ($P=0.196$, repeated measures ANOVA; Figure 9). Nasopharyngeal reflex-induced pressor responses, examined in the presence of methscopolamine, were also similar at all times in each group ($P=0.627$; Figure 9).

Orthostatic responses to 90° tilt

Intravenous peptide administration Peak orthostatic responses to 90° tilts performed at 0 (before), 30, 60 and 90 min after i.v. administration of vehicle, MVIIA (100 μg kg^{-1}) and CVID (100 μg kg^{-1}) are displayed in Figure 10. MAP and HR are shown at 0 s (just before tilt) and 5 s into the 1 min tilt (the average time point for the peak changes in MAP and HR). At 0 min, there was a tachycardia and pressor response to tilt of similar magnitude in all treatment groups. Treatments did not affect the tachycardic response to tilt at any time point ($P=0.414$, repeated measures ANOVA). However, the MAP responses to tilt were significantly different between treatments ($P=0.025$). MVIIA ($P=0.007$) and CVID ($P=0.039$) were each different from vehicle (repeated measures ANOVA). Changes in MAP in response to tilt at 30 min post-treatment were 14±6, −2±3 and −4±2 mmHg in vehicle, MVIIA and CVID groups, respectively ($n=4$ each; Figure 10).

However, there was a marked difference between the tilt responses 60 and 90 min after CVID compared with MVIIA treatment. For MVIIA, there was a sustained decrease in MAP at the start of these tilts (0 s) and MAP fell 3 mmHg within the first 5 s. In contrast, MAP had recovered towards the 0 min baseline value at 60 and 90 min post-CVID administration and the responses to tilt were slight increases of 3 mmHg at the 5 s point into the tilt. These data suggest that the blunted autonomic vasoconstrictor reflex to tilt recovered more quickly after CVID compared with treatment with MVIIA (Figure 10).

Intrathecal peptide administration Peak orthostatic responses to 90° tilts performed at 0 min (before), 30 min, 24 and 48 h after i.t. administration of vehicle, MVIIA (3 μg kg^{-1}) or CVID (3 μg kg^{-1}) are displayed in Figure 11. A peak rise in MAP and HR occurred in response to tilt at all experimental time points ($P=0.0001$ and $P=0.0006$, respectively; repeated measures ANOVA). These tachycardic and pressor responses to tilt were of similar magnitude in the three treatment groups ($P>0.05$).

Discussion

The principal findings of this study are that ω-conotoxin CVID, like ω-conotoxin MVIIA, is a potent inhibitor of N-

Table 3 Effect of intrathecal vehicle, ω-conotoxins MVIIA or CVID on baroreflex curve parameters in conscious rabbits

		Baroreflex curve parameter					
Treatment	*Time* (h)	*Average MAP* (mmHg)	*Average HR* (beats min^{-1})	*Lower HR plateau* (beats min^{-1})	*HR range* (beats min^{-1})	*MAP$_{50}$* (mmHg)	*Average gain* (beats min^{-1} mmHg^{-1})
Vehicle	0	73±4	201±11	166±8	149±11	67±4	−5.8±0.7
$n=4$	0.5	70±3	203±7	170±1	158±4	62±4	−5.8±0.2
	24	71±2	218±8	178±2	167±9	64±3	−6.7±0.9
	48	69±2	212±2	174±9	148±15	64±3	−7.2±1.4
MVIIA 3 μg kg^{-1}	0	75±5	211±12	178±13	158±7	70±5	−8.7±1.4
$n=4$	0.5	70±5	207±14	168±18	143±2	65±5	−6.3±1.0
	24	66±6	210±11	166±18	162±13	62±5	−9.1±2.7
	48	68±5	210±11	176±11	157±20	64±5	−11.1±4.2
CVID 3 μg kg^{-1}	0	72±1	209±11	176±9	152±11	68±2	−8.9±0.6
$n=5$	0.5	68±4	201±10	166±8	138±12	64±4	−6.8±0.3
	24	68±2	217±5	185±6	125±8	63±2	−6.0±1.3
	48	71±2	208±8	184±8	133±13	65±3	−6.8±1.1

Values are mean±s.e.mean. MAP, mean arterial pressure. HR, heart rate. MAP$_{50}$, MAP at half the HR range. *n*, number of animals. Average baroreceptor-heart rate reflex curve parameters obtained in conscious rabbits at times 0 h (before), 0.5, 24 and 48 h after intrathecal administration of vehicle (saline), ω-conotoxin MVIIA (MVIIA) or ω-conotoxin CVID (CVID).

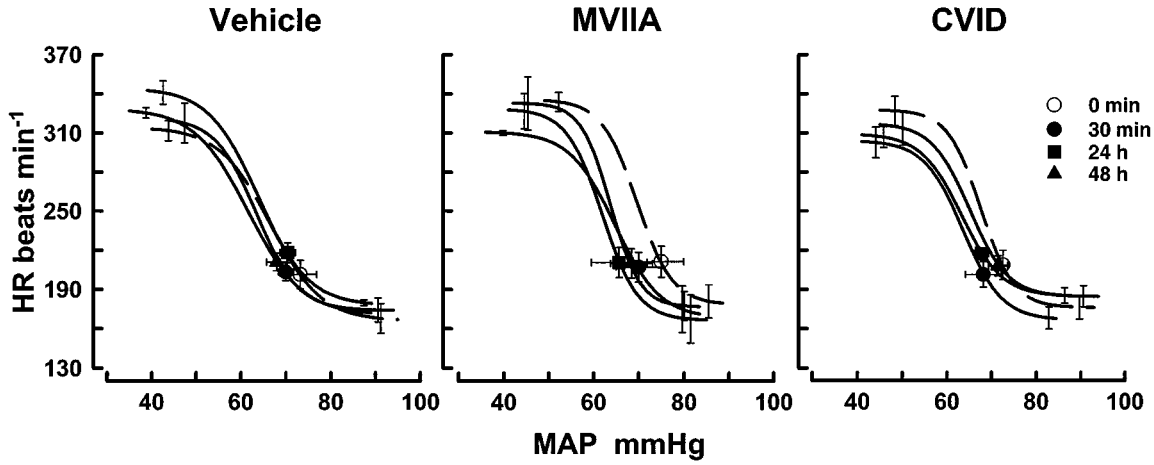

Figure 7 Average baroreceptor-heart rate reflex curves relating heart rate (HR) to mean arterial pressure (MAP) in conscious rabbits. Curves were constructed before (dashed line), 30 min, 24 h and 48 h after intrathecal administration of vehicle (0.9% saline; $n=4$), ω-conotoxin MVIIA 3 μg kg^{-1} ($n=4$) or ω-conotoxin CVID 3 μg kg^{-1} ($n=5$). The symbol on the curve represents the average resting values for HR and MAP. Error bars on the symbols are s.e.mean (those not visible are contained within the symbol) and those on the curves represent the s.e.mean of the lower HR plateau (right) and the HR range (left).

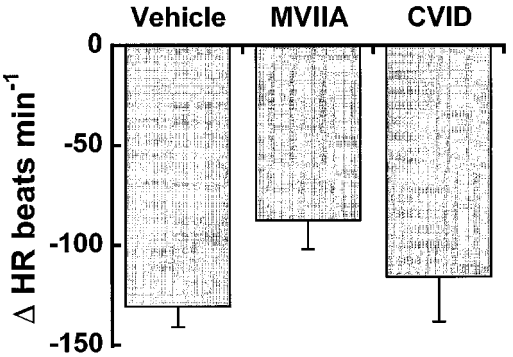

Figure 8 Change in heart rate (Δ HR) from baseline elicited by the nasopharyngeal reflex in conscious rabbits 30 min after intravenous administration of vehicle (saline; $n=6$), ω-conotoxin MVIIA 100 μg kg^{-1} (MVIIA; $n=6$) or ω-conotoxin CVID 100 μg kg^{-1} (CVID; $n=6$). Error bars are s.e.mean.

type calcium channels on sympathetic nerves with fast onset and offset of action in isolated tissue assays. In the conscious rabbit the haemodynamic effects of the sympatholytic peptides given intravenously on blood pressure, heart rate and the baroreceptor-heart rate reflex are equivalent (though less potent) to ω-conotoxin GVIA. CVID and MVIIA when administered intrathecally at 3 μg kg^{-1} have no autonomic or cardiovascular effects. CVID appears to be 10 fold weaker at inhibiting vascular sympathetic nerve responses *in vitro*, compared with its cardiac sympatholytic action, which may explain the much attenuated effect of CVID compared with MVIIA or GVIA in causing postural hypotension after intravenous administration.

In vitro assays

It was important to first compare the potencies of MVIIA and CVID with the now classical GVIA in isolated tissue assays at equilibrium. The experimental design chosen was to equilibrate tissues with the peptide concentration for 30 min (or 20 min for vas deferens) before reapplying the nerve stimulation. Although this cumulative design may have limitations, it does allow comparison within tissue and among peptides. In a recent comparison, incubation times for the peptides varied in rat isolated vas deferens assays from 5 min for MVIIA to 30 min for GVIA at each

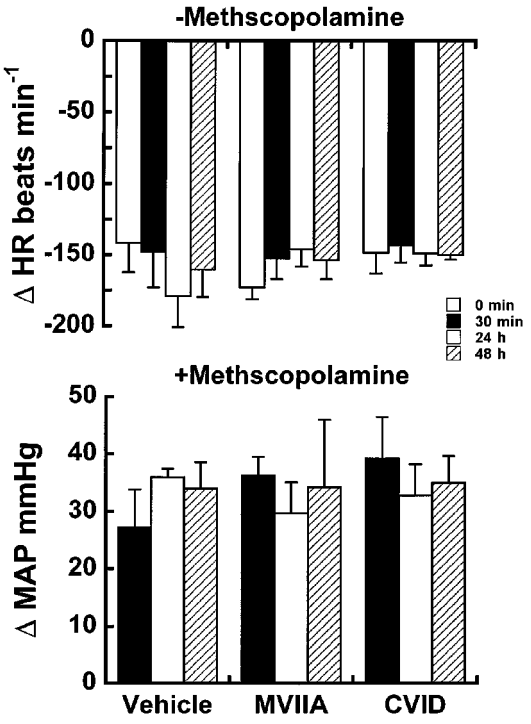

Figure 9 Changes in heart rate (Δ HR; absence (−) of methscopolamine) and mean arterial pressure (Δ MAP; in presence (+) of 50 μg kg^{-1} i.v. methscopolamine) from baseline elicited by the nasopharyngeal reflex in conscious rabbits at 0 min (before), 30 min, 24 and 48 h after intrathecal administration of vehicle (saline; $n=4$), ω-conotoxin MVIIA 3 μg kg^{-1} (MVIIA; $n=4$) or ω-conotoxin CVID 3 μg kg^{-1} (CVID; $n=5$). Error bars are s.e.mean.

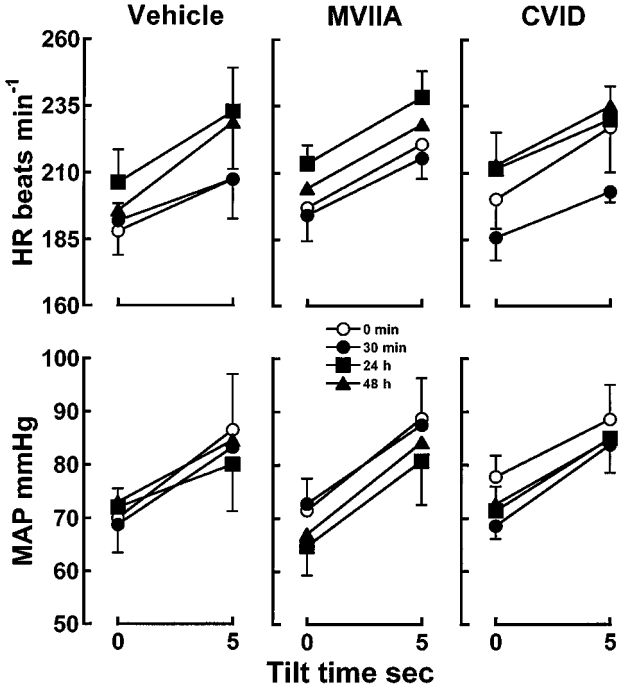

Figure 11 Peak orthostatic changes in heart rate (HR) and mean arterial pressure (MAP) in response to 90° tilt in conscious rabbits. Measurements were made at 0 s (just before the tilt) and 5 s (the peak change in HR or MAP). Orthostatic responses to tilt are shown before (0 min), 30 min, 24 and 48 h after intrathecal administration of vehicle (0.9% saline; $n=4$), ω-conotoxin MVIIA 3 μg kg^{-1} ($n=4$) or ω-conotoxin CVID 3 μg kg^{-1} ($n=5$). Error bars are s.e.mean; those that would overlap other symbols and/or lines are not shown for clarity.

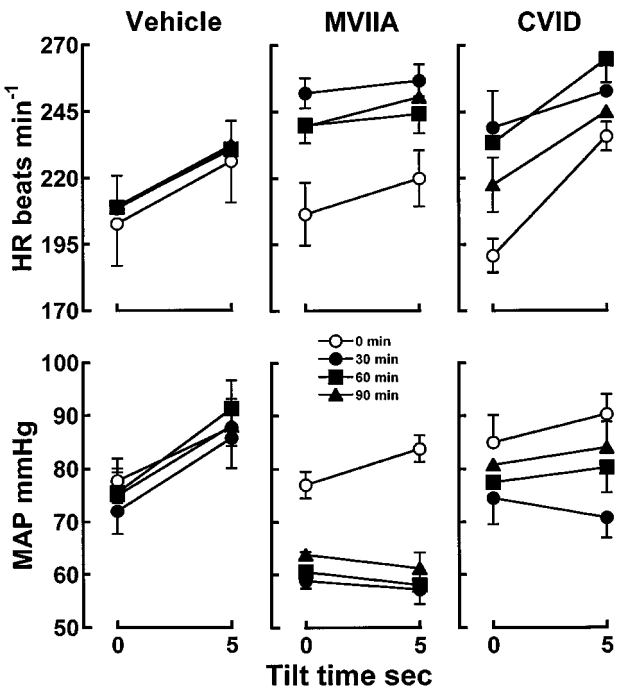

Figure 10 Peak orthostatic changes in heart rate (HR) and mean arterial pressure (MAP) in response to 90° tilt in conscious rabbits. Measurements were made at 0 s (just before the tilt) and 5 s (the peak change in HR or MAP). Orthostatic responses to tilt are shown before (0 min), 30, 60 and 90 min after intravenous administration of vehicle (0.9% saline; $n=4$), ω-conotoxin MVIIA 100 μg kg^{-1} (MVIIA; $n=4$) or ω-conotoxin CVID 100 μg kg^{-1} (CVID; $n=4$). Error bars are s.e.mean; those that would overlap other symbols and/or lines are not shown for clarity.

concentration. The pIC_{50} values were very similar to the values from the present work, i.e. GVIA 9.2±0.1 (versus 8.9±0.05 in this study) and MVIIA 9.0±0.1 (this study 9.0±0.07) (Sanger *et al*., 2000). The results show that the three peptides were equipotent in the three assays with one exception. CVID was 10 fold less potent in the mesenteric artery preparation compared with the vas deferens or atrium assay. Importantly, the sympathetic nerves were stimulated with parameters that were N-type voltage-operated calcium channel blocker-sensitive (100%) to avoid confounding the assay estimate of pIC_{50} with N-channel-resistant (perhaps P/Q channel-sensitive) responses (see Wright & Angus, 1996; Sanger *et al*., 2000).

These isolated tissue assays allowed a direct comparison of offset rates of CVID and MVIIA in three tissues. It appears that both peptides have a much faster onset and offset rate than GVIA (Figure 3 compared with GVIA in Flinn *et al*., 1999). The rapid onset after washout for 30 min and reapplication of the peptide indicates a lack of significant desensitization to MVIIA and CVID. The reason that GVIA should have such a prolonged on/off time may be related to structural factors and to access within the channel pore. For example, it was found recently that the offset rate of inhibition of the rat vas deferens twitch to GVIA was dramatically accelerated when a single amino acid at tyrosine 13 was substituted by its optical isomer D-tyrosine, although this substitution also caused some 17 fold loss of potency (Flinn *et al*., 1999).

Conscious rabbit experiments

Intravenous administration of GVIA has been shown to cause hypotension and tachycardia in the conscious rabbit

(Pruneau & Angus, 1990; Wright & Angus, 1995; 1997; Whorlow *et al*., 1998). The hypotension is due to a sympatholytic action which results in peripheral vasodilatation (Pruneau & Angus, 1990; Wright & Angus, 1995). The tachycardia is due to withdrawal of cardiac vagal efferent activity rather than a direct effect on the heart as the tachycardia still occurs in the presence of the β-adrenoceptor antagonist, propranolol (Whorlow *et al*., 1998). As GVIA and MVIIA both target the N-type calcium channel, it is not surprising that the peptides cause similar changes in cardiovascular parameters.

However, while the MVIIA-related peptides have a similar effect on the magnitude of MAP and heart rate change in the conscious rabbit, GVIA is more potent (by approximately 10–30 fold) but, consistent with its *in vitro* properties, has a slower onset and offset time. For example, it takes around 30–40 min for the peak changes in MAP and heart rate to be observed following GVIA (10 μg kg^{-1} i.v.; Wright & Angus, 1995) while following MVIIA (100 μg kg^{-1} i.v.) the peak changes occurred after 5–10 min and in the CVID group (also 100 μg kg^{-1} i.v.) the maximum effect was observed after 15 min. Some of the apparent delay in reaching the peak hypotensive effect was probably due to buffering from the renin-angiotensin system as recently shown for GVIA (Whorlow *et al*., 1998). In the current study, MAP and heart rate responses after MVIIA and CVID were returning towards baseline by 90 min, however following GVIA, the hypotension and tachycardia remain at a plateau for 3–4 h (Wright & Angus, 1995; 1997).

In the conscious rat, i.v. MVIIA was shown to cause hypotension within 30 s of administration (Bowersox *et al*., 1992). Higher doses of MVIIA resulted in a more profound hypotension which could be partially prevented by the administration of histamine H_1 and H_2 receptor antagonists. Thus, the hypotension in rats was explained by the sympatholytic effect of MVIIA combined with the induction of histamine release from mast cells (Bowersox *et al*., 1992). In the present study in the conscious rabbit, histamine H_1 and H_2 receptor antagonists, mepyramine and cimetidine, respectively, were given before MVIIA administration. There was no difference in the effect of MVIIA on MAP and heart rate either in the presence or absence of the histamine antagonists. Therefore, there does not appear to be a histamine-mediated component in the decrease in MAP caused by N-type calcium channel blockade in the conscious rabbit. This finding is consistent with the sparse distribution of tissue mast cells surrounding the microcirculation in the rabbit compared with a dense mast cell population in rats and mice (Riley, 1959). Similarly, but in contrast to the findings in the conscious rat of Bowersox *et al*. (1992), H_1, H_2 and H_3 receptor antagonism did not significantly affect the pIC_{50} for MVIIA in the rat right atrial assay.

Following MVIIA or CVID, there was a significant increase in the lower vagally-mediated and upper sympathetically-mediated components of the baroreflex curve. GVIA also has a similar effect on the baroreflex in conscious rabbits (Wright & Angus, 1995; 1997; Whorlow *et al*., 1998). However, the vagally-mediated bradycardia and sympathetically-mediated pressor response evoked by the nasopharyngeal reflex (White *et al*., 1974) was unaffected by MVIIA or CVID. The baroreflex and nasopharyngeal reflex are evoked by different stimuli and mediated *via* different afferent pathways but the same efferent pathway (i.e. the cardiac vagus). Therefore, it has been hypothesized that the selective vagolytic action of GVIA on the baroreflex may be due to an interaction with the afferent pathway of the reflex (Wright & Angus, 1995; 1997). Presumably this phenomenon also holds for MVIIA and CVID, consistent with N-type calcium channel blockade, or similar access kinetics.

Importantly, there is a clear difference between the effect of intravenous MVIIA and CVID on the orthostatic reflex. The hypotensive response following 90° head-up tilt was short-lived following CVID compared with the sustained effect following MVIIA, or previously published for GVIA (Hawkes *et al*., 1995; Wright & Angus, 1997; Wright *et al*., 2000). It is plausible that this weaker action of CVID on postural adaptation is related to its 10 fold weaker potency in the isolated artery assay and may point to some unique feature of this vascular N-type calcium channel.

N-type calcium channel blockers such as MVIIA, and CVID, have potential therapeutic applications in two main areas: (i) preventing neuronal damage following cerebral ischaemia; and (ii) in providing relief from pain (for review, see Shen *et al*., 2000). The intravenous administration of these N-type calcium channel blockers causes hypotension in the conscious rabbit, an undesirable side effect in the clinical setting. Considering that i.v. GVIA causes orthostatic hypotension in rabbits (Hawkes *et al*., 1995), and i.v. MVIIA has similar effects in humans at doses of 3.3–10 μg kg^{-1} 24 h^{-1} (McGuire *et al*., 1997), an unanswered question is whether intrathecal administration also has side effects such as hypotension, or causes central nervous system disturbances. In a case report (Brose *et al*., 1997), effective management of intractable brachial plexus avulsion pain in a human patient was obtained with continuous intrathecal infusion of MVIIA. In this case report it was possible to adjust the dose up to 48 ng kg^{-1} 24 h^{-1} to result in complete relief from pain without side effects such as nystagmus, blurred vision and dizziness which were observed at a higher dose (72 ng kg^{-1} 24 h^{-1}). Recently, Penn & Paice (2000) highlighted serious supraspinal and systemic adverse effects in several patients treated with intrathecal MVIIA at very high doses ranging between approximately 192 and 1800 ng kg^{-1} 24 h^{-1} (based on 70 kg body weight). Further, these effects required a long time for recovery suggesting that MVIIA may remain bound within tissue. Whether this was a consequence of the high doses administered is uncertain. In rabbits, central administration of GVIA requires 48 h to reach a maximum effect following a single i.c.v. bolus of drug (Whorlow *et al*., 1994).

In conclusion, the present study shows that MVIIA and CVID cause similar falls in MAP, increase in heart rate and attenuation of some autonomic reflexes following intravenous, but not intrathecal, administration in conscious rabbits. The faster onset/offset times of these peptides *in vivo* would be an advantage over other longer-lasting N-type calcium channel blockers such as GVIA in allowing more rapid adjustment of dose. The comparison of CVID and MVIIA in an isolated small artery assay *in vitro* and on the tilt reflex *in vivo* indicate that CVID may relatively spare reflex sympathetic vasoconstrictor responses. This finding may indicate that there are some N-type calcium channel differences on sympathetic nerves that may offer therapeutic advantages for selective antagonists.

This study was supported by a grant from AMRAD Operations Pty. Ltd. The authors wish to thank Mr Peter Coles and Mr Mark Ross-Smith for expert technical assistance with the *in vitro* experiments.

References

ANGUS, J.A., BROUGHTON, A. & MULVANY, M.J. (1988). Role of α-adrenoceptors in constrictor responses of rat, guinea-pig and rabbit small arteries to neural activation. *J. Physiol. (Lond.),* **403,** 495 – 510.

BOWERSOX, S.S., SINGH, T., NADASDI, L., ZUKOWSKA-GROJEC, Z., VALENTINO, K. & HOFFMAN, B.B. (1992). Cardiovascular effects of ω-conopeptides in conscious rats: mechanisms of action. *J. Cardiovasc. Pharmacol.,* **20,** 756 – 764.

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 – 259.

FLINN, J.P., PALLAGHY, P.K., LEW, M.J., MURPHY, R., ANGUS, J.A. & NORTON, R.S. (1999). Roles of key functional groups in ω-conotoxin GVIA. Synthesis, structure and functional assay of selected peptide analogues. *Eur. J. Biochem.,* **262,** 447 – 455.

HAWKES, A.L., ANGUS, J.A. & WRIGHT, C.E. (1995). ω-Conotoxin GVIA and prazosin, but not felodipine, cause postural hypotension in rabbits. *Clin. Exp. Pharmacol. Physiol.,* **22,** 711 – 716.

HEAD, G.A. & ADAMS, M.A. (1992). Characterization of the baroreceptor heart rate reflex during development in spontaneously hypertensive rats. *Clin. Exp. Pharmacol. Physiol.,* **19,** 587 – 597.

HEAD, G.A. & McCARTY, R. (1987). Vagal and sympathetic components of the heart rate range and gain of the baroreceptor-heart rate reflex in conscious rats. *J. Auton. Nerv. Syst.,* **21,** 203 – 213.

HEY, J.A., DEL PRADO, M., EGAN, R.W., KREUTNER, W. & CHAPMAN, R.W. (1992). Inhibition of sympathetic hypertensive responses in the guinea-pig by prejunctional histamine H_3-receptors. *Br. J. Pharmacol.,* **107,** 347 – 351.

ISHIKAWA, S. & SPERELAKIS, N. (1987). A novel class (H_3) of histamine receptors on perivascular nerve terminals. *Nature,* **327,** 158 – 160.

LEW, M.J., FLINN, J.P., PALLAGHY, P.K., MURPHY, R., WHORLOW, S.L., WRIGHT, C.E., NORTON, R.S. & ANGUS, J.A. (1997). Structure-function relationships of ω-conotoxin GVIA: Synthesis, structure, calcium channel binding, and functional assay of alanine-substituted analogues. *J. Biol. Chem.,* **272,** 12014 – 12023.

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 mammalian calcium channel subtypes. *J. Biol. Chem.,* in press.

LUDBROOK, J. (1994). Repeated measurements and multiple comparisons in cardiovascular research. *Cardiovasc. Res.,* **28,** 303 – 311.

McGUIRE, D., BOWERSOX, S., FELLMANN, J.D. & LUTHER, R.R. (1997). Sympatholysis after neuron-specific, N-type, voltage-sensitive calcium channel blockade: first demonstration of N-channel function in humans. *J. Cardiovasc. Pharmacol.,* **30,** 400 – 403.

MULVANY, M.J. & HALPERN, W. (1997). Contractile properties of small arterial resistance vessels in spontaneously hypertensive and normotensive rats. *Circ. Res.,* **41,** 19 – 26.

NIELSEN, K.J., SCHROEDER, T. & LEWIS, R. (2000). Structure-activity relationships of ω-conotoxins at N-type voltage-sensitive calcium channels. *J. Mol. Recognit.,* **13,** 55 – 70.

PENN, R.D. & PAICE, J.A. (2000). Adverse effects associated with the intrathecal administration of ziconotide. *Pain,* **85,** 291 – 296.

PRUNEAU, D. & ANGUS, J.A. (1990). ω-Conotoxin GVIA, the N-type calcium channel inhibitor, is sympatholytic but not vagolytic: consequences for hemodynamics and autonomic reflexes in conscious rabbits. *J. Cardiovasc. Pharmacol.,* **16,** 675 – 680.

RILEY, J.F. (1959). *The Mast Cells*. London: Livingstone.

SANGER, G.J., ELLIS, E.S., HARRIES, M.H., TILFORD, N.S., WARDLE, K.A. & BENHAM, C.D. (2000). Rank-order inhibition by ω-conotoxins in human and animal autonomic nerve preparations. *Eur. J. Pharmacol.,* **388,** 89 – 95.

SHEN, G.S., LAYER, R.T. & McCABE, R.T. (2000). Conopeptides: From deadly venoms to novel therapeutics. *Drug Discov. Today,* **5,** 98 – 106.

SNEDECOR, G.W & COCHRAN, W.G. (1989). Statistical Methods. pp. 254 – 272. Ames: Iowa State University Press.

WHITE, S.W., McRITCHIE, R.J. & FRANKLIN, D.L. (1974). Autonomic cardiovascular effects of nasal inhalation of cigarette smoke in the rabbit. *Aust. J. Exp. Biol. Med. Sci.,* **52,** 111 – 126.

WHORLOW, S.L., ANGUS, J.A. & WRIGHT, C.E. (1994). The effects of central administration of ω-conotoxin GVIA on cardiovascular parameters and autonomic reflexes in conscious rabbits. *Clin. Exp. Pharmacol. Physiol.,* **21,** 865 – 873.

WHORLOW, S.L., ANGUS, J.A. & WRIGHT, C.E. (1998). Endogenous angiotensin II and bradykinin delay and attenuate the hypotension after N-type calcium channel blockade in conscious rabbits. *J. Cardiovasc. Pharmacol.,* **32,** 951 – 961.

WRIGHT, C.E. & ANGUS, J.A. (1996). Effects of N-, P- and Q-type neuronal calcium channel antagonists on mammalian peripheral neurotransmission. *Br. J. Pharmacol.,* **119,** 49 – 56.

WRIGHT, C.E. & ANGUS, J.A. (1983). Haemodynamic response to ketanserin in rabbits with Page hypertension: comparison with prazosin. *J. Hypertens.,* **1,** 183 – 190.

WRIGHT, C.E. & ANGUS, J.A. (1995). Hemodynamic and autonomic reflex effects of chronic N-type Ca^{2+} channel blockade with ω-conotoxin GVIA in conscious normotensive and hypertensive rabbits. *J. Cardiovasc. Pharmacol.,* **25,** 459 – 468.

WRIGHT, C.E. & ANGUS, J.A. (1997). Prolonged cardiovascular effects of the N-type Ca^{2+} channel antagonist ω-conotoxin GVIA in conscious rabbits. *J. Cardiovasc. Pharmacol.,* **30,** 392 – 399.

WRIGHT, C.E., ANGUS, J.A. & KORNER, P.I. (1987). Vascular amplifier properties in renovascular hypertension in conscious rabbits. Hindquarter responses to constrictor and dilator stimuli. *Hypertension,* **9,** 122 – 131.

WRIGHT, C.E., HAWKES, A.L. & ANGUS, J.A. (2000). Postural hypotension following N-type Ca^{2+} channel blockade is amplified in experimental hypertension. *J. Hypertens.,* **18,** 65 – 73.

YAKSH, T.L. & RUDY, T.A. (1976). Chronic catheterization of the spinal subarachnoid space. *Physiol. Behav.,* **17,** 1031 – 1036.

(Received July 3, 2000
Revised September 7, 2000
Accepted September 13, 2000)