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Neurotoxic activity of venom from the Australian Eastern mouse spider (*Missulena bradleyi*) involves modulation of sodium channel gating

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1 Mouse spiders represent a potential cause of serious envenomation in humans. This study examined the activity of *Missulena bradleyi* venom in several *in vitro* preparations. Whilst female *M. bradleyi* venom at doses up to 0.05 μl ml^{-1} failed to alter twitch or resting tension in all preparations used, male venom (0.02 and 0.05 μl ml^{-1}) produced potent effects on transmitter release in both smooth and skeletal neuromuscular preparations.

2 In the mouse phrenic nerve diaphragm preparation, male *M. bradleyi* venom (0.02 μl ml^{-1}) caused rapid fasciculations and an increase in indirectly evoked twitches.

3 Male venom (0.02 and 0.05 μl ml^{-1}) also caused a large contracture and rapid decrease in indirectly evoked twitches in the chick biventer cervicis muscle, however had no effect on responses to exogenous ACh (1 mM) or potassium chloride (40 mM). In the chick preparation, contractile responses to male *M. bradleyi* venom (0.05 μl ml^{-1}) were attenuated by (+)-tubocurarine (100 μM) and by tetrodotoxin (TTX, 1 μM). Both actions of male *M. bradleyi* venom were blocked by *Atrax robustus* antivenom (2 units ml^{-1}).

4 In the unstimulated rat vas deferens, male venom (0.05 μl ml^{-1}) caused contractions which were inhibited by a combination of prazosin (0.3 μM) and P_{2X}-receptor desensitization (with α,β-methylene ATP 10 μM). In the rat stimulated vas deferens, male venom (0.05 μl ml^{-1}) augmented indirectly evoked twitches.

5 Male venom (0.1 μl ml^{-1}) causes a slowing of inactivation of TTX-sensitive sodium currents in acutely dissociated rat dorsal root ganglion neurons.

6 These results suggest that venom from male *M. bradleyi* contains a potent neurotoxin which facilitates neurotransmitter release by modifying TTX-sensitive sodium channel gating. This action is similar to that of the δ-atracotoxins from Australian funnel-web spiders.
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Abbreviations: α,β-mATP, α,β-methylene adenosine 5-triphosphate; DRG, dorsal root ganglion; TTX, tetrodotoxin

Introduction

Mouse spiders (Araneae: Actinopodidae) belong to the infraorder Mygalomorphae and are medium to large, powerfully built trap-door spiders with a wide-spread distribution over mainland Australia. There are currently 11 recognized species of mouse spider in the genus *Missulena* (Main, 1985, 1996; Faulder, 1995) with the larger species growing to a body length of 35 mm or 20 mm for female and male spiders, respectively. Male *M. bradleyi* are smaller (up to 16 mm body length) and less stocky than the female (up to 25 mm body length) and have a trapezium-shaped lighter patch, from white and pale blue to mauve in colour, on the anterior dorsal surface of the abdomen. The abdomen of the female is brown to almost black and the carapace of both sexes is a shiny black. Mature males have the unusual habit, for mygalomorphs, of seeking a mate during day-light hours.

Despite the extensive distribution and diurnal habits of the male, bites by mouse spiders are not common or at least not commonly reported. Of the few cases of envenomation known, several have consisted of systemic reactions (Faulder, 1993). The most severe case involved a 19-month-old girl who within 30 min of being bitten on the finger by a male Eastern mouse spider (*Missulena bradleyi*) began vomiting and lost consciousness. Subsequent symptoms included heavy perspiration, difficulty in breathing and tachycardia. The administration of funnel-web spider antivenom, raised against male *Atrax robustus* venom, surprisingly produced a dramatic improvement in her condition and a full recovery was made following a second dose of antivenom (Underhill, 1987).

The aim of this study was to examine the venom from *M. bradleyi* to determine any possible neurotoxic activity and to determine the effectiveness of funnel-web spider antivenom in neutralizing the actions of mouse spider venom.

Methods

Spider collection and venom preparation

Spiders were collected from the Gosford and Newcastle regions along the central coast of the state of New South Wales and maintained in the laboratory in individual containers. Male spiders were aggravated and the venom expressed at the fang tips aspirated into plastic tubing attached

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to a microsyringe. Female spiders were less aggressive therefore electrostimulation (20–30V, 20 Hz) was applied across the chelicera, using electrode gel to enhance electrical contact. Expressed venom was collected from the fang tips. Venom was diluted in distilled water and immediately stored frozen (−20°C). Venom concentrations are expressed as volume (μl) of wet venom per ml.

Mouse phrenic nerve diaphragm

Adult male BALB/c mice were killed and the hemidiaphragms, with the phrenic nerve intact, removed. Preparations were attached to tissue holders with in-built electrodes, mounted in 5 ml organ baths, and maintained at 37°C under 1 g resting tension. Hemidiaphragms were stimulated *via* the phrenic nerve at supramaximal voltages (0.1 Hz, 0.2 ms) using a Grass S88 stimulator. Indirect stimulation was confirmed by abolition of twitches by (+)-tubocurarine (10 μM). After this procedure the tissue was equilibrated for at least 30 min before the addition of venom.

Chick isolated biventer cervicis muscle preparation

Biventer cervicis muscles were removed from male chicks (4–10 days old), mounted in 5 ml organ baths and maintained at 34°C under 1 g resting tension. Twitches were evoked by stimulating the motor nerve (supramaximal voltage, 0.2 ms, 0.1 Hz) *via* silver electrodes connected to a Grass S88 stimulator. Nerve mediated (indirectly evoked) twitches were confirmed by the addition of (+)-tubocurarine (10 μM). To elicit direct stimulation of the preparation the electrodes were lowered to the level of the muscle belly (supramaximal voltage, 2 ms, 0.1 Hz). To ensure the resulting twitches were not partially mediated by nerve stimulation these experiments were carried out in the continual presence of (+)-tubocurarine (10 μM). In the absence of electrical stimulation, responses to exogenous acetylcholine (1 mM, 30 s) and KCl (40 mM, 30 s) were obtained prior to the addition of venom and at the conclusion of the experiment. Preparations were allowed to equilibrate for at least 30 min with continuous stimulation before the addition of venom.

Rat isolated vas deferens

Vasa deferentia were removed from adult male Sprague-Dawley rats and bisected to give prostatic and epididymal segments as previously described (Rash *et al*., 1998). Tissues were mounted on electrodes (prostatic) or wire tissue holders (epididymal), placed in 5 ml organ baths under 1 g resting tension and maintained at 32°C. Vas deferens were equilibrated for 45 min, during which time prostatic segments were stimulated by regular field pulses (supramaximal voltage, 0.3 ms, 0.2 Hz) applied transmurally from a Grass S88 stimulator.

Experimental conditions

All isolated preparations were maintained in a physiological saline solution of the following composition (mM): NaCl 118.4; KCl 4.7; $MgSO_4$ 1.2; KH_2PO_4 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25; D-glucose 11.1 and bubbled continuously with 95% O_2, 5% CO_2. Isometric contractions were measured *via* a Grass transducer (FTO3) connected to a Grass polygraph (Model 79D) or Powerlab/400 system. Where required, antagonists were in contact with the tissue for an initial period of 30 min and for 5 min on subsequent additions before the re-testing of agonists or venom.

Electrophysiological studies

Acutely dissociated dorsal root ganglion (DRG) neurons were prepared from 4–12 day old Wistar rats and maintained in short-term primary culture using the method described by Nicholson *et al*. (1994). Voltage-clamp recordings were made using an AxoPatch 200A patch-clamp amplifier (Axon Instruments, Foster City, CA, U.S.A.) and whole-cell patch-clamp techniques (Hamill *et al*., 1981). Micropipettes were pulled from borosilicate glass capillary tubing (Corning 7052 Glass, Warner Instruments) and had d.c. resistances of 0.8–2.0 MΩ.

To record macroscopic sodium currents, micropipettes were filled with a solution of the following composition (in mM): CsF 135; NaCl 10; HEPES 5; with the pH adjusted to 7.0 with 1M CsOH. The external bathing solution contained (in mM): NaCl 30; $MgCl_2$ 1; $CaCl_2$ 1.8; CsCl 5; KCl 5; D-glucose 25; HEPES 5; tetraethylammonium (TEA) chloride 20; tetramethylammonium chloride 70; with the pH adjusted to 7.4 with 1M TEA hydroxide.

The osmolarity of all solutions was adjusted to 290–300 mOsm with sucrose to reduce osmotic stress. The external solution was applied to the perfusion chamber *via* a gravity-fed perfusion system, and the flow rate maintained at 0.5 ml min^{-1} using a Gilmont flowmeter (Barrington, IL, U.S.A.). Data were recorded at room temperature (22–25°C) which did not fluctuate more than 1°C during the course of an experiment. In voltage-clamp experiments the holding potential was −80 mV and the sodium concentration in the external solution was reduced to 30 mM to improve series resistance compensation and to avoid saturation in the recording system (Ogata *et al*., 1989). Inverted voltage-clamp command pulses were applied to the bath through a Ag/AgCl pellet/3M KCl-agar bridge. The liquid junction potential between internal and external solutions was approximately −6 mV, and all data was compensated for this value.

Large round light DRG cells with diameters of 20–40 μM were selected for experiments. Larger cells from older animals tended to express fast tetrodotoxin (TTX)-sensitive sodium currents whilst smaller cells tended to express predominantly slow TTX-resistant sodium currents (Roy & Narahashi, 1992). The predominant sodium channel subtype present in each cell was determined using a modified steady-state sodium channel inactivation voltage protocol, taking advantage of the separation of steady-state inactivation curves for TTX-sensitive and TTX-resistant sodium channels (Roy & Narahashi, 1992). Briefly, a 1 s conditioning prepulse to −130 mV, sufficient to force all sodium channels into the resting state, was followed by a 50 ms test pulse to −10 mV, resulting in maximal sodium current activation during the test pulse. This was compared with the sodium current elicited by the same 50 ms test pulse to −10 mV this time preceeded by a conditioning prepulse potential to −50 mV. This conditioning prepulse was sufficient to inactivate all available TTX-sensitive sodium channels leaving only TTX-resistant sodium channels available for activation during the test pulse. The ratio of the test currents recorded at prepulse potentials of −50 and −130 mV provides a quantitative measure of the proportion of TTX-resistant sodium currents in the total whole-cell sodium current (data not shown). Only those cells which exhibited less than 10% TTX-resistant sodium current, as determined from differences in this steady-state sodium channel inactivation profile, were used to determine the

actions of toxins on TTX-sensitive sodium currents. In those experiments that assessed the actions of *M. bradleyi* venom on TTX-resistant currents, 200 nM TTX was applied in the external solution to eliminate any residual TTX-sensitive sodium currents. After breaking through the membrane, experiments did not commence for a period of 20–30 min to allow for the complete block of calcium and potassium currents and any fast time-dependent shifts in steady-state inactivation. The experiments used in this study were rejected if there were large leak currents or currents showed signs of poor space clamping such as an abrupt activation of currents upon relatively small depolarizing pulses.

Stimulation and recording were both controlled by a pClamp© data acquisition system (Axon Instruments). Data were filtered at 5 kHz (4 pole low-pass Bessel filter) and digital sampling rates were between 15 and 25 kHz depending on voltage protocol length. Leakage and capacitive currents were digitally subtracted with *PP/4* procedures (Bezanilla & Armstrong, 1977) and series resistance compensation was >80% for all cells. Data analyses were performed off-line following completion of the experiment. Mathematical curve fitting employed algorithms available in KaleidaGraph® for Macintosh using a non-linear least squares method.

To examine the effect of male *M. bradleyi* venom on the current/voltage relationship, families of sodium currents were evoked by 50 ms depolarizations, from −80 to +70 mV, in 5 mV steps applied every 10 s from a holding potential of −80 mV in the absence and presence of venom.

The curve fits for the I/V data were obtained using the following equation:

$$I_{Na} = g_{max}(1 - (\frac{1}{1 + \exp[(V - V_{1/2})/s]}))(V - V_{rev}) \quad (1)$$

where I_{Na} is the amplitude of the peak sodium current at a given test potential, V, g_{max} is the maximal sodium conductance, $V_{1/2}$ is the voltage at half-maximal activation, s is the slope factor and V_{rev} is the reversal potential.

Drugs and chemicals

The following drugs and chemicals were used: acetylcholine chloride, α,β-methylene adenosine 5-triphosphate (α,β-mATP), noradrenaline bitartrate, prazosin hydrochloride, (+)-tubocurarine chloride, tetrodotoxin (TTX) were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.). Funnel-web spider antivenom was obtained from CSL Ltd. (Melbourne, Australia). Stock solutions of drugs were made up in distilled water unless otherwise stated. The stock solution and subsequent dilutions of noradrenaline were made in catecholamine diluent (0.9% NaCl, 0.0156% NaH_2PO_4, 0.004% ascorbic acid, w v^{-1}). Prazosin was dissolved in methanol then made up to 50% methanol, 50% distilled water.

Statistics

Paired student's *t*-tests were used to compare venom/agonist responses before and after antagonists in the same tissue, and unpaired Student's *t*-tests performed on venom/agonist responses in the presence/absence of antagonists in different tissues. A two-way repeated measures analysis of variance (ANOVA) followed by an appropriate *post hoc* test (Bonferroni) was used to compare the response of venom over time before and after antagonists. Values of $P<0.05$ were considered significant. Data are expressed as mean±s.e.mean.

Results

Effects on the mouse phrenic nerve diaphragm

In the mouse phrenic nerve diaphragm preparation male *M. bradleyi* venom (0.02 μl ml^{-1}) caused fasciculations and a significant increase (453±135% peak increase at $t=4.8\pm1.8$ min, $n=5$, $P<0.05$, paired Student's *t*-test) in indirectly evoked twitch height (Figure 1a,b), the increase in twitch height and fasciculations persisted for the time of exposure to male venom and was only partially reversed after repeated washing with venom free solution over 30 min. In contrast, venom from female *M. bradleyi* (0.02 μl ml^{-1}) had no effect on either resting tension or twitch height (4.4±3.4% peak change in twitch height, $n=4$, $P>0.05$, paired Student's *t*-test) (Figure 1a,b).

Effects on the chick isolated biventer cervicis muscle

Male *M. bradleyi* venom (0.05 μl ml^{-1}) caused a large contracture with fasciculations in the chick biventer cervicis muscle, whereas venom (0.05 μl ml^{-1}) from female *M. bradleyi*

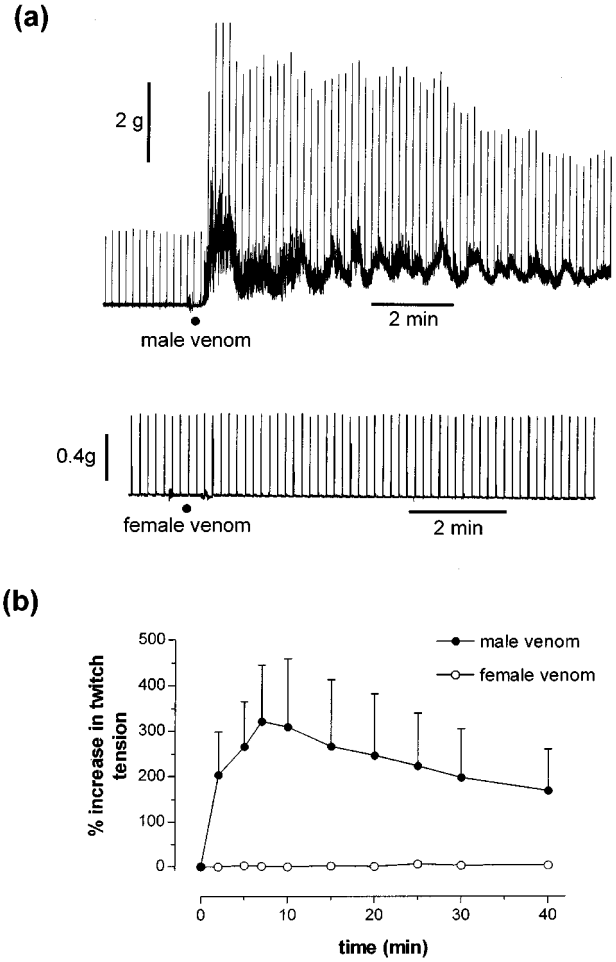

Figure 1 (a) Male *M. bradleyi* venom (0.02 μl ml^{-1}) induces fasciculations and increases evoked twitch amplitude (upper trace), while venom (0.02 μl ml^{-1}) from the female had no effect (lower trace) in the indirectly stimulated (0.1 Hz, 0.2 ms, supramaximal voltage) mouse isolated phrenic nerve diaphragm. (b) Graph illustrating the effect of male and female *M. bradleyi* venom (0.02 μl ml^{-1}) on indirectly elicited twitch amplitude in the mouse phrenic nerve diaphragm, $n=5$. Venom was added at $t=0$ and left in contact with the tissue for the duration of the experiment.

had no effect (Figure 2a). In the indirectly stimulated biventer cervicis muscle, male *M. bradleyi* venom (0.02 and 0.05 μl ml^{-1}) caused a large contracture (Figure 4a) in conjunction with a rapid decrease in twitch height (Figure 2b,c) but had no significant effect on responses to ACh (1 mM: 1.6$\pm$0.4 g before venom, 1.6$\pm$0.5 g after venom) or KCl (40 mM: 1.5$\pm$0.5 g before venom, 1.2$\pm$0.4 g after venom) (n=4, P>0.05, paired Student's t-test). Female *M. bradleyi* venom (0.05 μl ml^{-1}) had no significant effect on either basal tension or twitch height (Figure 2c). In the directly stimulated biventer cervicis muscle neither male or female *M. bradleyi* venom (0.02 μl ml^{-1}) had any effect on twitch height or resting tension (male, 87.8$\pm$4; female, 103.4$\pm$1.2, control, 99$\pm$3.2, per cent original twitch height 30 min after addition of venom n=3, P>0.05 unpaired Student's t-test: compared to 24.7$\pm$8.8% for male venom 30 min after addition to the indirectly stimulated preparation). The contracture induced by male *M. bradleyi* venom (0.05 μl ml^{-1}) was significantly inhibited by the sodium channel antagonist tetrodotoxin (TTX, 1 μM) (Figure 3). The skeletal muscle nicotinic receptor antagonist (+)-tubocurarine (100 μM) significantly inhibited the contractile responses to ACh (1 mM; 1.9$\pm$0.3 before vs 0.03$\pm$0.01 after (+)-tubocurarine, n=4, P<0.01 paired Student's t-test) and male *M. bradleyi* venom (0.05 μl ml^{-1}; 3.5$\pm$0.4 alone vs 0.6$\pm$0.1 in presence of (+)-tubocurarine, P<0.001 unpaired Student's t-test) (Figure 3) but not KCl (40 mM, 2.3$\pm$0.2 before vs 1.9$\pm$0.3 after (+)-tubocurarine, n=4, P>0.05 paired Student's t-test). Prior exposure of the tissue (for 10 min) to funnel-web spider antivenom (2 units ml^{-1}) prevented the contraction and the attenuation of twitches caused by male *M. bradleyi* venom (0.02 μl ml^{-1}) in the indirectly stimulated biventer cervicis (n=4, Figure 4).

Effects on the rat isolated vas deferens

In the epididymal segment of the rat vas deferens, male but not female *M. bradleyi* venom (0.05 μl ml^{-1}) caused a prolonged contraction with fasciculations (Figure 5a). The α_1-adrenoceptor antagonist prazosin (0.3 μM) in combination with P_{2X} receptor desensitization (with α, β-mATP, 10 μM) attenuated the responses to noradrenaline (2 μM: 1.0$\pm$0.3 g alone, 0.0$\pm$0.0 g in presence of antagonism), α,β-mATP (1 μM: 0.5$\pm$0.2 g alone, 0.0$\pm$0.0 g in presence of antagonism) and male *M. bradleyi* venom (0.05 μl ml^{-1}: 0.7$\pm$0.2 alone, 0.08$\pm$0.01 g in presence of antagonism, n=3, Figure 5b). In the rat stimulated vas deferens (prostatic segment) electrically evoked twitches were potentiated by male (62.1$\pm$3.7% increase,

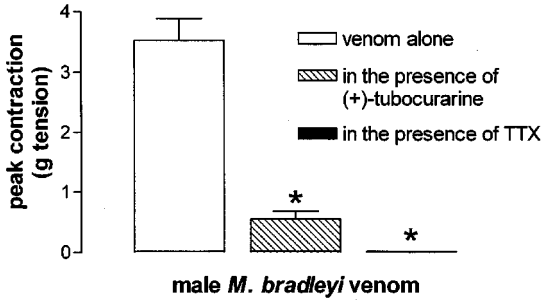

Figure 3 The peak contraction of the chick biventer cervicis muscle caused by male *M. bradleyi* venom (0.05 μl ml^{-1}, n=10) is significantly attenuated by (+)-tubocurarine (100 μM, n=4) and TTX (1 μM, n=4). (*P<0.001 different from venom alone, unpaired Student's t-test).

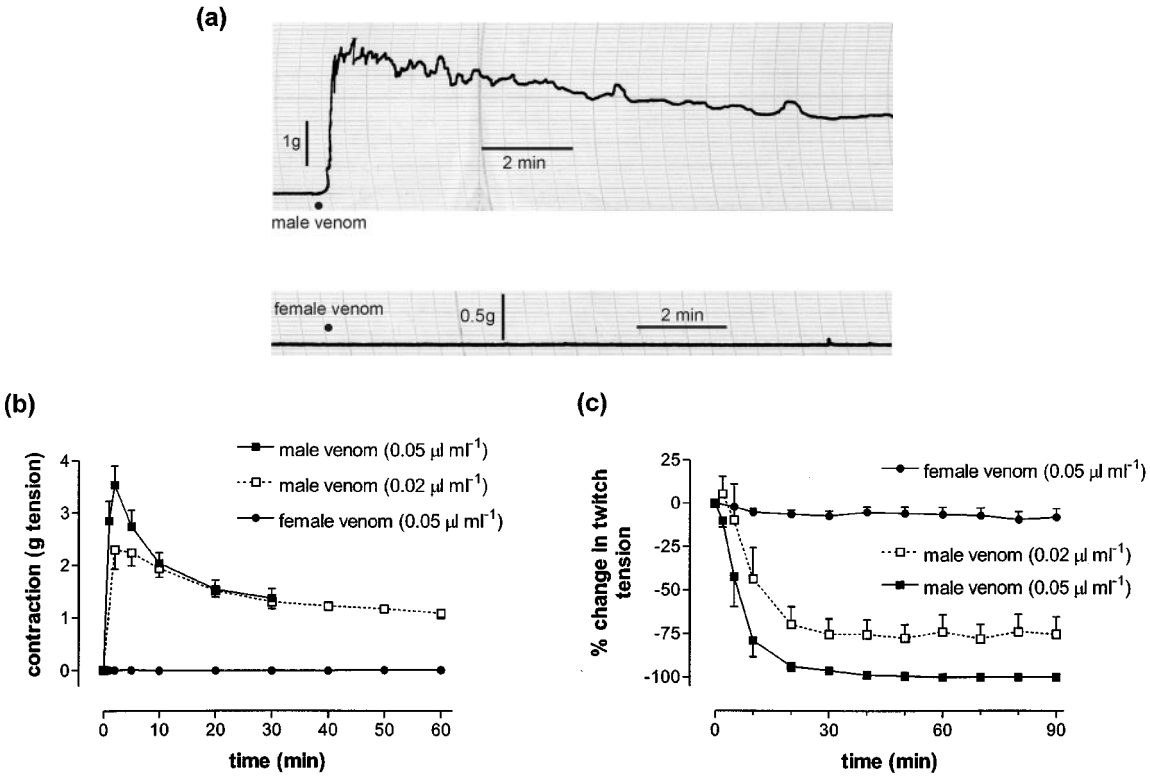

Figure 2 (a) Male (upper trace) but not female (lower trace) *M. bradleyi* venom (0.05 μl ml^{-1}) causes a large contracture in the chick unstimulated biventer cervicis muscle. (b) Effect of male and female *M. bradleyi* venom on resting tension in the chick biventer cervicis muscle, n=4–10. (c) Effect of male and female *M. bradleyi* venom on twitch tension in the indirectly stimulated (0.1 Hz, 0.2 ms) chick biventer cervicis muscle, n=4. Venom was added at t=0 and left in contact with the tissue for the duration of the experiment.

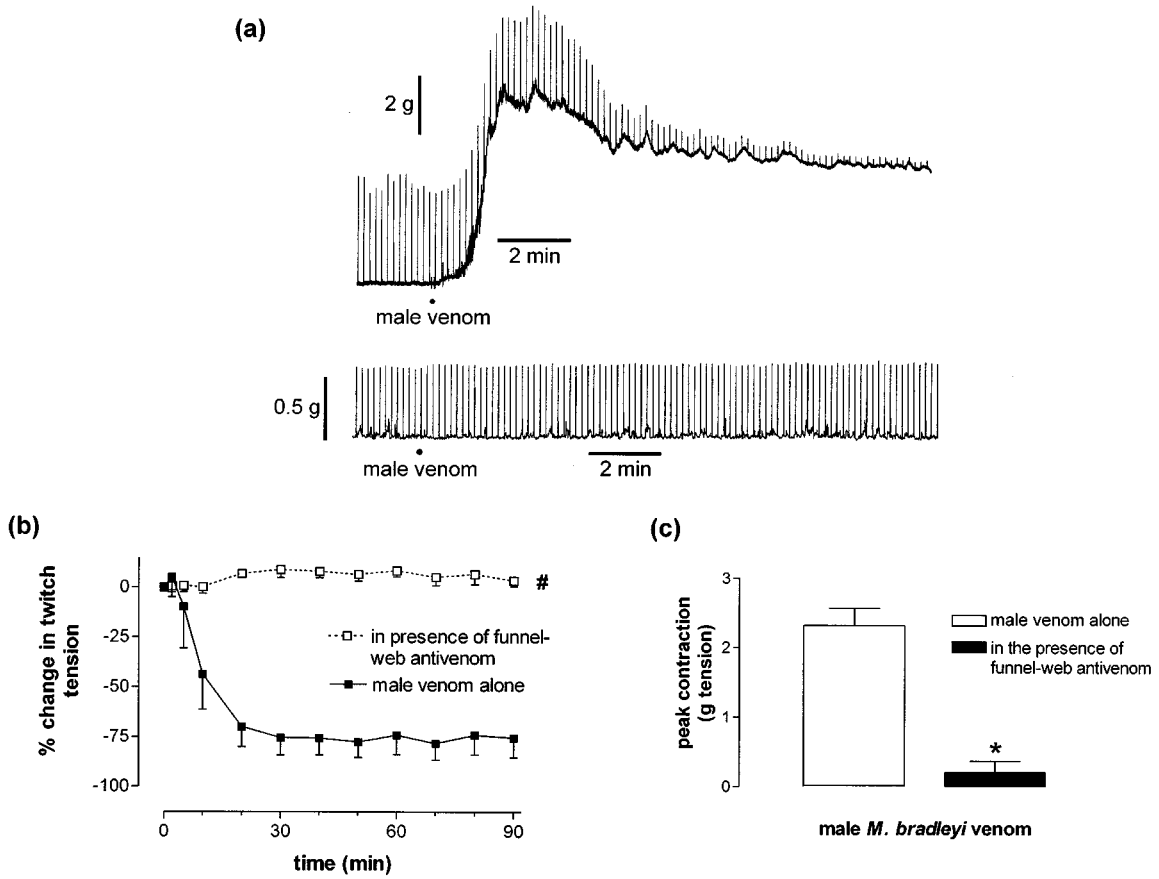

Figure 4 Neutralization of the effects of male *M. bradleyi* venom by funnel-web spider antivenom in the chick isolated biventer cervicis preparation. (a) Trace showing the response of indirectly stimulated (0.1 Hz, 0.2 ms) muscle to male *M. bradleyi* venom (0.02 μl ml^{-1}) alone (top trace) and following 10 min incubation with funnel-web spider antivenom (2 units ml^{-1}) (lower trace). Effect of funnel-web spider antivenom (2 units ml^{-1}) on the response of indirectly stimulated muscle to male *M. bradleyi* venom (0.02 μl ml^{-1}), on twitch amplitude (b) and peak contraction (c), $n=4$. #$P<0.001$ different from venom alone, two-way repeated measures ANOVA; *$P<0.001$ different from venom alone, unpaired Student's *t*-test).

$n=4$) but not female (4.2 ± 1.5% increase, $n=3$) *M. bradleyi* venom (0.05 μl ml^{-1}) (Figure 5c).

Effects on DRG sodium currents

Under voltage-clamp conditions, male *M. bradleyi* venom exerted a selective concentration-dependent reduction in the rate of TTX-sensitive sodium current inactivation. Figure 6a shows the effect of male *M. bradleyi* venom on TTX-sensitive sodium currents. At 0.1 μl ml^{-1} male *M. bradleyi* venom did not significantly affect peak TTX-sensitive sodium current amplitude ($n=4$, $P>0.05$, paired Student's *t*-test). However, male *M. bradleyi* venom slowed the rate of the TTX-sensitive sodium current inactivation as evidenced by a sustained current during depolarizing test potentials (26.9 ± 2.9% of control peak sodium current, $n=4$). This action on sodium channel inactivation is similar to δ-atracotoxins from Australian funnel-web spider venoms (Nicholson *et al*., 1994; 1998). Like δ-atracotoxins the slowing of current inactivation produced by male *M. bradleyi* venom was not reversible, even after prolonged washing with venom-free solution.

Previous studies have also shown that δ-atracotoxins shift the threshold of activation of TTX-sensitive sodium currents in the hyperpolarizing direction (Nicholson *et al*., 1994; 1998). Comparison of the effect of male *M. bradleyi* venom (0.1 μl ml^{-1}) revealed similar hyperpolarizing shifts in the threshold of activation by approximately 10 mV (Figure 6g). The hyperpolarizing shift in the activation voltage suggested that male *M. bradleyi* venom affects the voltage dependence of activation and accordingly the effects on the voltage dependence of sodium conductance were examined. Data were fitted using equation (1) in the Methods. Male *M. bradleyi* venom caused a significant shift in the voltage midpoint ($V_{1/2}$) from -41.3 ± 8.2 mV to -49 ± 8.5 mV ($P<0.01$, $n=4$, paired Student's *t*-test). There was, however, no significant change in the slope factor. Figure 6g also shows that these changes occurred in the absence of significant changes in reversal potential which were only slightly decreased by 2.5 ± 0.2 mV ($P>0.3$, $n=4$).

Consistent with δ-atracotoxins, and in marked contrast to its action on TTX-sensitive sodium currents, male *M. bradleyi* venom at doses up to 0.25 μl ml^{-1} failed to significantly alter either the amplitude, time course or voltage-dependence of activation of TTX-resistant sodium currents (Figure 6 right hand column).

Discussion

The rapid contracture and fasciculations seen in the rat vas deferens and chick biventer cervicis muscle to male *M. bradleyi* venom were inhibited by antagonism of the endogenous excitatory neurotransmitters suggesting that the venom causes the release of these substances. In the chick biventer cervicis preparation male *M. bradleyi* venom also caused a decrease in nerve mediated twitches but did not antagonize the response to

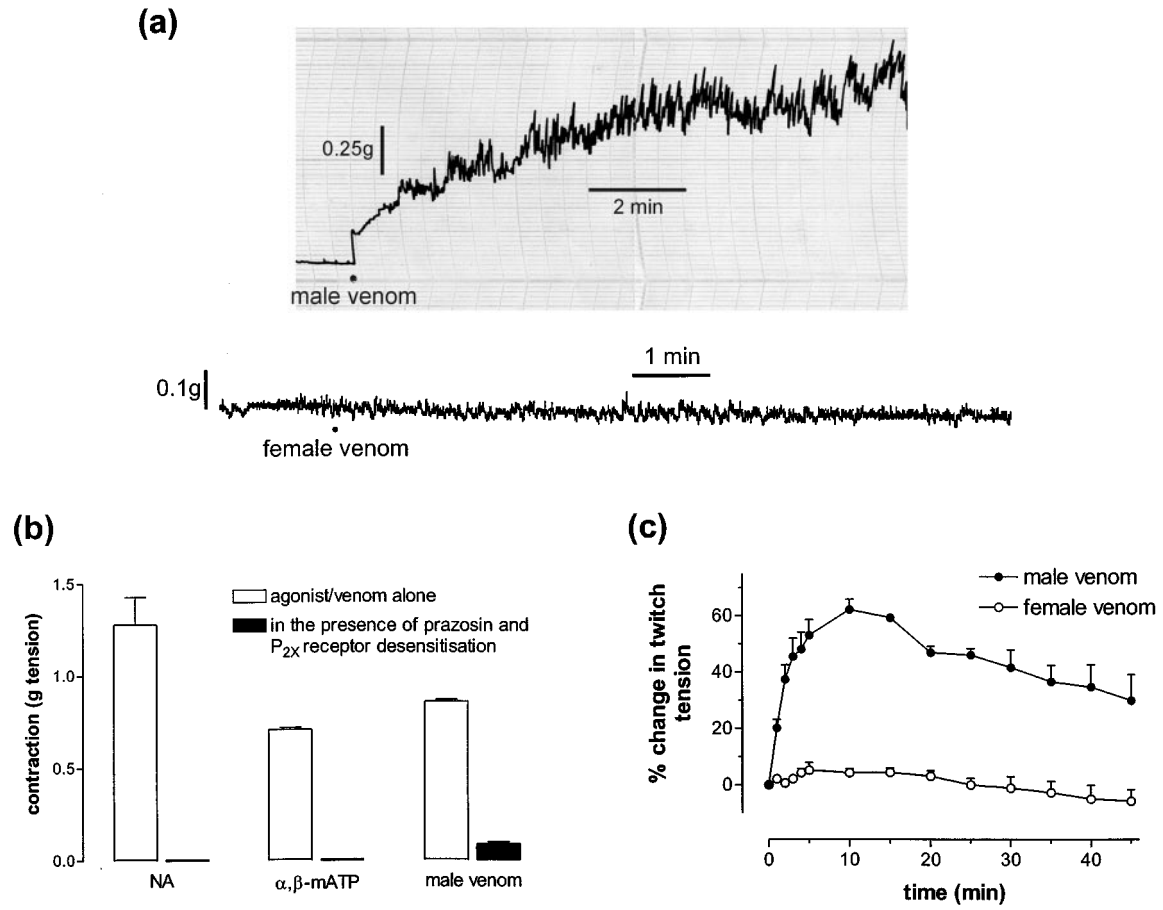

Figure 5 (a) Traces showing the contractile response of rat vas deferens (epididymal segment) to male but not female *M. bradleyi* venom (0.05 μl ml^{-1}). (b) Effect of prazosin (0.3 μM) and P_{2X} receptor desensitization (α,β-mATP 10 μM) on responses to NA (2 μM), α,β-mATP (1 μM) and male *M. bradleyi* venom (0.05 μl ml^{-1}), $n=3$. (c) Male but not female *M. bradleyi* venom (0.05 μl ml^{-1}) causes an increase in twitch amplitude in the rat stimulated vas deferens (prostatic segment; 0.2 Hz, 0.3 ms), $n=3$. Venom was added at $t=0$ and left in contact with the tissue for the remainder of the experiment.

ACh or have any effect on directly stimulated preparations. The response to male *M. bradleyi* venom in the chick biventer preparation was inhibited by the sodium channel blocker TTX which has been reported to have no effect on the response to exogenous ACh and carbachol in this preparation (Marshall *et al*., 1979). These results suggest that male *M. bradleyi* venom is acting presynaptically rather than postsynaptically. Furthermore, TTX is known to block nerve conduction but have no effect on the release of transmitter from presynaptic terminals (Evans, 1972; Gong *et al*., 1995). This suggests that male *M. bradleyi* venom is acting at the level of the nerve fibre to cause neurotransmitter release rather than at the terminal, as do the venoms of *Latrodectus hasselti* and *L. mactans*, the contracture responses to which are unaffected by TTX (Einhorn & Hamilton, 1973; Pinto *et al*., 1974).

Gage & Spence (1977) found that in the mouse phrenic nerve diaphragm *A. robustus* venom caused fasciculations and prolonged contractures that were inhibited by both tubocurarine and TTX and suggested that the site of action was the nerve fibre. In the chick biventer cervicis muscle a crude venom extract produced fasciculations and potentiated indirectly evoked twitches yet caused no loss of function of the tissue (Sutherland, 1978). In the present study, male *M. bradleyi* venom attenuated indirect stimulation of the chick preparation however, augmented indirectly evoked twitches in the mouse hemidiaphragm. The latter effect is similar to that observed for atraxotoxin. The differing effects of atraxotoxin and male *M. bradleyi* venom on twitch height may be due to species differences with respect to sensitivity to the neurotoxin in male *M. bradleyi* venom.

Symptoms of envenomation by the male funnel-web spider (*A. robustus*) consist of nausea, vomiting, abdominal pain, profuse sweating, salivation, lachrimation and dyspnoea. Hypertension, generalized muscle fasciculation and coma may follow the initial symptoms (Sutherland, 1978). These symptoms closely resemble those observed following envenomation by a male Eastern mouse spider in a 19-month-old girl (Underhill, 1987). Given the overlapping distributions of *M. bradleyi* and *A. robustus*, their likeness to the untrained eye and the apparent similarity of their envenomation syndromes, it is possible that some cases of presumed, but unconfirmed, funnel-web spider bites are actually due to male *M. bradleyi*. The similar symptoms of envenomation and the fact that the 19-month-old girl recovered fully after the administration of antivenom raised against the male Sydney funnel-web spider *A. robustus*, an unrelated spider, suggests the presence of a neurotoxin possibly with a similar action to robustoxin found in male *A. robustus* venom. Indeed, neurotoxic activity was found in the venom of male *M. bradleyi* in all preparations tested, however this activity was absent in venom from the female spider. This is in contrast with the venom of female *M. occatoria* which was found to be lethal to suckling mice (LD_{50} of 0.1 mg kg^{-1}, compared to 0.25 mg kg^{-1} for crude atraxotoxin) and caused muscle fasciculations in the rat

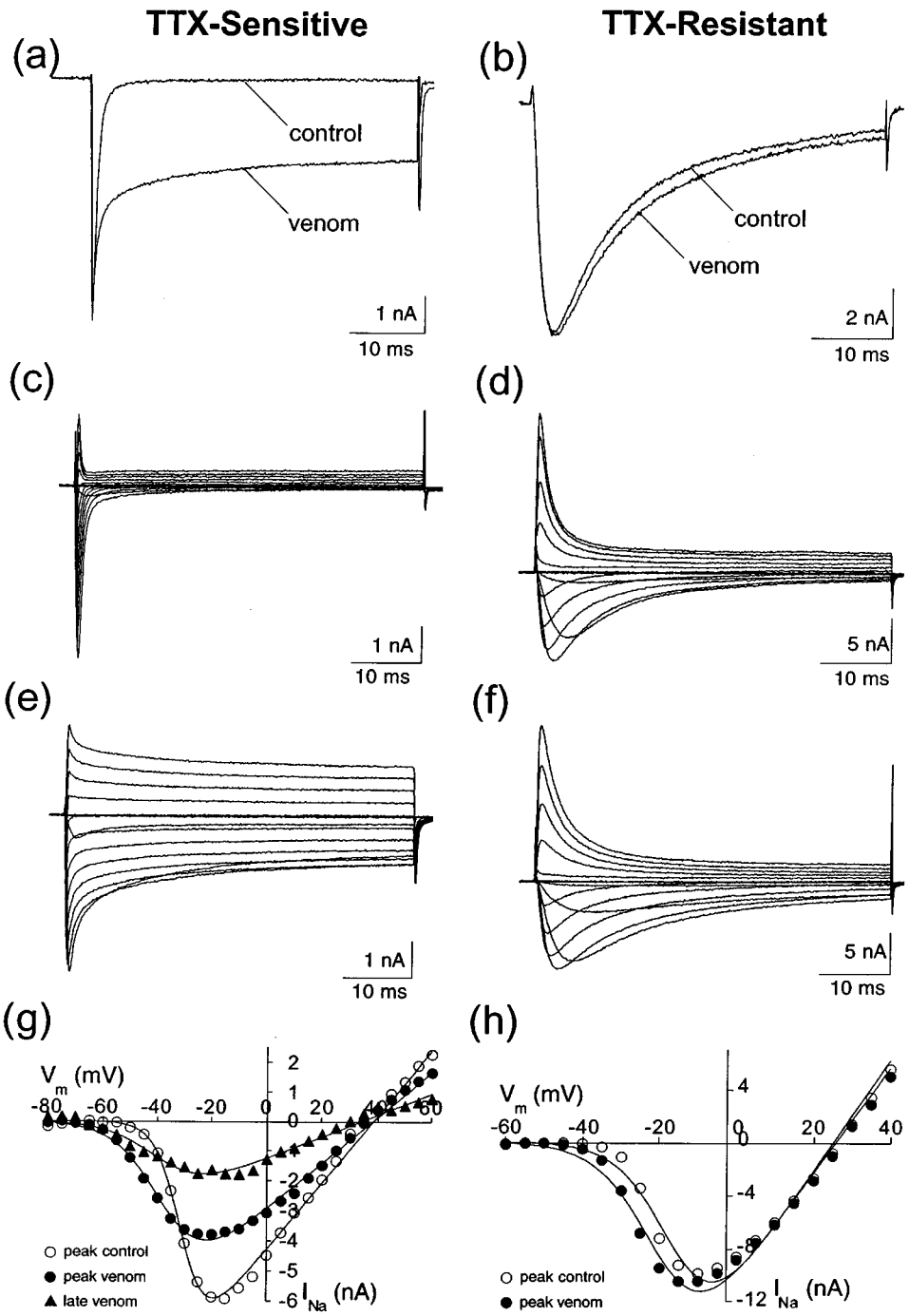

Figure 6 Effects of male *M. bradleyi* venom (0.1 μl ml^{-1}) on TTX-sensitive sodium currents and TTX-resistant sodium currents, recorded from rat dorsal root ganglion cells. (a,b) Superimposed current traces recorded following a 50 ms depolarization to -10 mV before, and 10 min after, venom application. Note the slowed inactivation kinetics of TTX-sensitive sodium currents in (a). (c–f) Typical families of sodium currents before (c and d) and after (e and f) a 10 min application of male *M. bradleyi* venom. Sodium currents were evoked by 50 ms depolarizations from -80 to $+70$ mV in 5 mV steps applied every 10 s from a holding potential of -80 mV. Only traces in 10 mV steps are shown for clarity. (g,h) Peak and late current/voltage (I/V) relationships are graphed before, and 10 min after, application of *M. bradleyi* venom. Late currents were measured at the end of each 50 ms depolarizing test pulse. TTX-sensitive sodium currents in a, c, e and g were recorded from the same cell and all TTX-resistant sodium currents (b, d, f and h) were recorded from a different cell, in TTX.

phrenic nerve diaphragm (Sutherland, 1979; 1983). However, as Faulder (1993) points out, at that time there was no reliable method for identifying female *Missulena* spiders, therefore, there may have been juvenile male spiders in the specimens from which venom was obtained.

Recently two neurotoxins (robustoxin and versutoxin, now δ-atracotoxin-Ar1 and -Hv1a, respectively) from the funnel-web spiders *A. robustus* and *Hadronyche versuta* have been isolated and pharmacologically characterized (Nicholson *et al*., 1994; 1998). In rat DRG cells δ-atracotoxins-Ar1 and -Hv1a selectively and dose-dependently act on TTX-sensitive sodium channels to slow or remove sodium current inactivation. Experiments with crude male *M. bradleyi* venom in DRG cells show similar selective effects on macroscopic sodium currents to those of δ-atracotoxins-Ar1 and -Hv1a, suggesting that it also modulates the gating and kinetics of TTX-sensitive sodium channels. These findings together with the effectiveness of *A. robustus* antivenom

against male *M. bradleyi* venom *in vitro*, in the present study, and in the clinical case mentioned earlier suggest that venom from male *M. bradleyi* contains a potent neurotoxin very similar in activity to δ-atracotoxins from Australian funnel-web spiders.

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