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Inhibition of Neuronal Ca^{2+} Channel Currents by the Funnel Web Spider Toxin ω-Aga-IA

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SUMMARY

Ca^{2+} channel currents were recorded from cultured rat dorsal root ganglion neurons and cerebellar granule cells using the whole-cell recording variant of the patch clamp technique. ω-Aga-IA, a toxin purified from the venom of the American funnel web spider, *Agelenopsis aperta*, markedly inhibited high threshold barium currents (I_{Ba}) when applied at 10 nM concentration. The low threshold T-type current activated at $V_C = -30$ mV and the outward (Ca^{2+} channel) current activated at +120 mV were significantly less sensitive to ω-Aga-IA. ω-Conotoxin GVIA (1 μM) inhibited I_{Ba} irreversibly. In contrast, the action of ω-Aga-IA was partially reversed 5 min after its removal. The voltage-activated calcium current (I_{Ca}) was inhibited by ω-Aga-IA in a manner different from I_{Ba}. I_{Ca} measured at the end of a 100-msec voltage step command was reduced to a greater extent than the peak current. The residual I_{Ca} following application of ω-Aga-IA was a fast transient current. ω-Aga-IA did not inhibit voltage-activated sodium currents from dorsal root ganglion neurons in the absence of tetrodotoxin. ω-Aga-IA abolished the dihydropyridine (+)-202-791-sensitive L-type current component of I_{Ba}. We conclude that ω-Aga-IA is a very potent inhibitor of neuronal voltage-activated Ca^{2+} channel currents and that it may prove to be a useful tool in the characterization and isolation of Ca^{2+} channels.

An increase in intracellular Ca^{2+} concentration underlies many important cellular processes, including release of neurotransmitter, channel activation, and modulation of enzyme activity. In neurons, as in other cell types, a major source of the Ca^{2+} signal is Ca^{2+} entering the cell from the extracellular environment through voltage-activated Ca^{2+} channels. Several types of voltage-activated Ca^{2+} channels have been described, which are high voltage activated (L- and N-types) or low voltage activated (T-type) (1–3). The classification of these Ca^{2+} channels is based on biophysical and pharmacological characteristics. However, identifying the contribution made by different Ca^{2+} channel types to the whole-cell current has proven difficult, because of overlap in the voltage dependence of activation and inactivation. Pharmacological tools appear for the most part not to be selective for the different types of voltage activated Ca^{2+} channels and often have variable actions on different cell types. For example, 1,4-dihydropyridines enhance or inhibit L-type currents (2) but have recently also been shown to interact with T-type currents (4). However, 1,4-dihydropyridine agonists have been useful in isolating L-type currents, which can be measured from the slowly deactivating tail current (5). Inorganic blockers (Cd^{2+}, Ni^{2+}) preferentially inhibit different types of voltage-activated Ca^{2+} channels but are not selective (2, 6). Finally, the toxin ω-CgTx, isolated from the marine snail *Conus geographus* (7), has been used in the study of voltage-activated Ca^{2+} channels. The selectivity of this toxin is still far from clear and interactions with N-, L-, and T-type currents have been reported (5, 8, 9), although ω-CgTx only partially inhibited T-type currents and this action was fully reversible, whereas the action on high threshold components was irreversible (8).

Organisms have evolved toxins that are often potent and in some cases are selective in the way in which they interact with constituent proteins of ion channels. A toxin isolated and purified from the venom of the American funnel web spider, *Agelenopsis aperta*, has been investigated in this study, because it may provide a tool to investigate the functional roles played by voltage-activated Ca^{2+} channels. The venom from *A. aperta* contains a multitude of toxins, which are currently classified into three groups, (a) polyamine α-agatoxins, (b) polypeptide μ-agatoxins, and (c) polypeptide ω-agatoxins (10, 11). Preliminary evidence suggests that the polypeptide ω-Aga-IA inhibits voltage-activated Ca^{2+} currents recorded from DRG neurons (12). ω-Aga-IA also blocks neuromuscular transmission at the insect neuromuscular junction and inhibits Ca^{2+} action poten-

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ABBREVIATIONS: ω-CgTx; ω-conotoxin GVIA; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; TTX, tetrodotoxin; DRG, dorsal root ganglion; GTPγS, guanosine 5′-*O*-(3-thio)triphosphate.

tials in insect neurons (13). The present study was carried out to investigate the action of ω-Aga-IA on neuronal Ca^{2+} channel currents and to determine whether this toxin has a selective action on a particular component of the whole-cell current.

Materials and Methods

Dissociated cultures. DRG neurons were dissected from 3-day old rats, dissociated, and plated on polyornithine-laminin-coated coverslips. The cultures were maintained in a serum-free defined medium (14, 15) for between 1 to 3 weeks. Cerebellar granule cells were dispersed from cerebella from 6 day-old rats, as previously described (16). Cells were plated on 2 cm^2 coverslips, at 5×10^6 cells/coverslip, and grown in modified Eagle's medium containing 10% heat-inactivated horse serum, 50 IU/ml penicillin, 50 μg/ml streptomycin, 25 μl/ml chick embryo extract (Flow), 39 mM glucose, 2 mM glutamine, and 23 mM KCl. The cells were incubated at 37° in humidified air containing 5% CO_2. After 48 hr, fluorodeoxyuridine (80 μM) was added to the culture medium to reduce the proliferation of nonneuronal cells. The culture medium was renewed every 3–4 days, and cells were used between 7 and 10 days in culture.

Electrophysiological experiments. The whole-cell recording technique (17) was used to study Ca^{2+} channel currents carried by either Ba^{2+} (I_{Ba}) or Ca^{2+} (I_{Ca}). Low resistance (1–3 MΩ) patch pipettes were used, and cells were voltage clamped with an Axoclamp-2a switching voltage clamp amplifier, operated at a sampling rate of 20–30 kHz.

Experiments were performed at room temperature and currents were evoked at a frequency of 0.03 Hz. The cells were allowed to equilibrate for 5 min following disruption of the membrane patch between the pipette and cytoplasm and entry into the whole-cell recording configuration. ω-Aga-IA or other agents were applied by continuous low pressure ejection (less than 1 psi, ~7 kPa) from a micropipette (tip diameter of 10 μm) placed about 100 μm from the cell. Bath application of ω-Aga-IA yielded very variable results; this may be due to the toxin binding to plastic, glass, and the culture substrate. (+)-(*S*)-202-791 (Sandoz) was dissolved at 10 mM in 70% ethanol and diluted in recording medium immediately before use. The final concentration of ethanol was 0.035%. Data were stored using a FM tape recorder and digitized at 2 kHz for analysis. Ca^{2+} channel currents are shown following digital subtraction of scaled linear leakage and capacitance currents, except where stated. This is particularly important for the study of outward currents activated by large depolarizations, because of substantial leak currents contributing to varying degrees in recordings from different cells. Leak currents generated by negative steps immediately before activation of outward currents were averaged and scaled to give the leak current produced by a large step. This leak was subtracted from the total outward current to give the net outward current produced by ions passing through voltage-activated channels. All data are given as mean ± standard error.

Solutions. The standard recording medium contained (in mM): NaCl, 130; KCl, 3; $MgCl_2$, 0.6; $NaHCO_3$, 1.0; HEPES, 10; glucose, 4; tetraethylammonium bromide, 25; TTX, 0.0025; and either $BaCl_2$, 2.5, or $CaCl_2$, 5. The solution was adjusted to pH 7.4 with NaOH and to 320 mOsM by addition of sucrose, I_{Na} was recorded using this Ba^{2+}-containing solution but in the absence of TTX. Patch electrodes were filled with a solution containing (in mM): cesium acetate, 140; $CaCl_2$, 0.1; EGTA, 1.1; $MgCl_2$, 2; ATP, 2; and HEPES, 10. The pCa of this solution was 8.0. The pH was adjusted to 7.2 with Tris and the osmolarity to 310 mOsM with sucrose. The actions of ω-CgTx (Peninsula Laboratories Inc.) were compared with those of ω-Aga-IA. ω-Aga-IA, a 66-amino acid polypeptide toxin, was purified from the venom of the funnel web spider, *A. aperta*, by multistep fractionation using reverse phase high performance liquid chromatography (11). Toxin purity was confirmed by UV spectra, amino acid composition, and microsequencing analysis (11). Samples (2 nmol) of purified toxin were vacuum evaporated to dryness in 1.5-ml Eppendorf centrifuge tubes and were stored under argon. Samples were resuspended as stock solutions in 200 μl of distilled water (10 μM) and were stored at −20° between experiments. Test solutions were made immediately before experiments by appropriate dilution of the stock solution into recording medium.

Results

Effect of ω-Aga-IA on DRG and cerebellar neuron Ba^{2+} currents. ω-Aga-IA, applied at concentrations between 10 nM and 1 μM, markedly inhibited the high voltage-activated Ba^{2+} current (I_{Ba}) recorded from cultured rat DRG neurons. The toxin (10 nM) inhibited the maximum I_{Ba} activated from V_H (holding potential) of −80 mV at V_c (clamp potentials) between 0 and +10 mV by 80 ± 7% and 87 ± 6% ($n = 9$), for the peak current and the current measured at the end of a 100-msec step, respectively. Under control conditions, the mean maximum I_{Ba} was 2.17 ± 0.44 nA and inactivated during a 100-msec voltage step command to 1.70 ± 0.42 nA; the current was reduced to 0.46 ± 0.16 nA and inactivated to 0.18 ± 0.07 nA by 10 nM ω-Aga-IA (applied for 5 min). The mean maximum I_{Ba} is the average of the largest currents that could be activated in the DRG neurons at V_c between 0 and 20 mV. The inhibitory action of ω-Aga-IA occurred without any shift in the voltage dependence of I_{Ba} and without any effect on the leak current, which was linear between −160 and −80 mV (Fig. 1A). I_{Ba} was also reduced by ω-Aga-IA when recorded from freshly replated cells, which have no processes.[2] ω-Aga-IA also inhibited the low threshold T-type current but to a lesser extent than the high threshold current. The T-type current was reduced by ω-Aga-IA over a time course of 3 to 5 min; application of the toxin for an additional 10 min did not inhibit the current further. As previously described, the low threshold T-type current was observed in about 50% of DRG cells (6). Application of 10 nM ω-Aga-IA for 5 min inhibited the peak T-type current activated from $V_H = -90$ mV at $V_c = -30$ mV by 46 ± 5% ($n = 6$), reducing the current from 0.70 ± 0.14 nA to 0.40 ± 0.10 nA (Fig. 1B). Control experiments showed that side fractions collected before or after high pressure liquid chromatographic elution of ω-Aga-IA had no effect on I_{Ba} in DRG cells when applied at dilutions of 1:100 in recording medium, compared with the toxin fraction, which was diluted 1:2500 to give 10 nM (Fig. 1C). ω-Aga-IA also inhibited I_{Ba} activated in cultured cerebellar neurons (Fig. 1D). The maximum high threshold I_{Ba} was reduced from 0.24 ± 0.06 nA to 0.05 ± 0.04 nA ($n = 4$) by 10 nM ω-Aga-IA. The mean percentage of inhibition was 85 ± 7%, suggesting that ω-Aga-IA was able to inhibit I_{Ba} recorded from cerebellar and DRG neurons to an equal extent.

Outward movement of monovalent ions (Cs^+ and K^+) through Ca^{2+} channels was observed during large depolarizing steps to $V_c = +70$ to +120 mV, as previously described (18, 19). The purpose of studying the actions of pharmacological agents on outward Ca^{2+} channel currents was to ensure that genuine inhibition of I_{Ca} had occurred, rather than a shift in voltage dependence. ω-Aga-IA (10 nM) inhibited the mean outward Ca^{2+} channel current by 32 ± 8% ($n = 8$), reducing the maximum net outward current activated at $V_c = +120$ mV from 1.83 ± 0.32 nA to 1.27 ± 0.31 nA (Fig. 2A). Some recovery of both inward and outward Ca^{2+} channel currents was observed following application of 10 nM ω-Aga-IA. Five minutes after removal of the pressure pipette containing ω-Aga-IA, following toxin

[2] R. H. Scott, preliminary data.

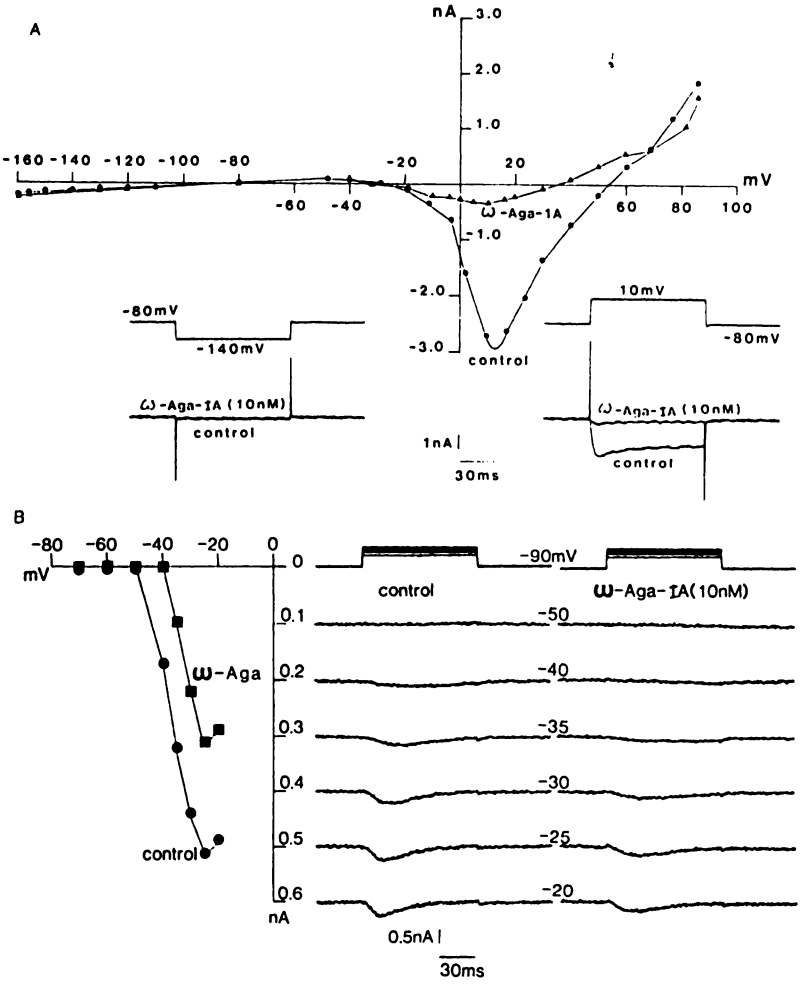
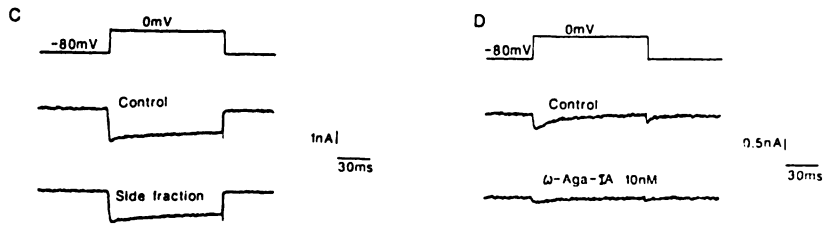


Fig. 1. Effect of ω-Aga-IA (10 nM) on I_{Ba} and leak current. A, *I-V* relationship, recorded from a DRG neuron (without leak subtraction), showing inhibition of control I_{Ba} (●) by a 5-min application of ω-Aga-IA (▲). No low threshold T-type current is present in this cell. The outward Ca^{2+} channel currents activated at potentials positive to 70 mV are also reduced by ω-Aga-IA. The leak current activated between −90 mV and −160 mV is unaffected by ω-Aga-IA and the null potential was unchanged at 70 mV. *Left inset traces*, the leak currents at −140 mV are unchanged from control currents, compared with the overlying current in the presence of ω-Aga-IA. *Right inset traces*, inhibition of I_{Ba} activated from V_H = −80 mV by a 90-mV depolarizing voltage step command to V_c = 10 mV by ω-Aga-IA. B, *I-V* relationship of low threshold T-type currents (leak subtracted) recorded from DRG neurons held at V_H = −90 mV under control conditions (●) and in the presence of ω-Aga-IA for 5 min (■). *Traces* show T-type currents activated between V_c = −50 mV and −20 mV and modest inhibition by 10 nM ω-Aga-IA. C, Side fraction, diluted 1:100 in recording medium, did not inhibit the high threshold I_{Ba} activated at 0 mV in DRG neurons. D, High threshold I_{Ba} recorded from cerebellar neurons activated from V_H = −80 mV by 100-msec voltage step commands to 0 mV. *Traces* show inhibition of I_{Ba} by 10 nM ω-Aga-IA (applied for 5 min).



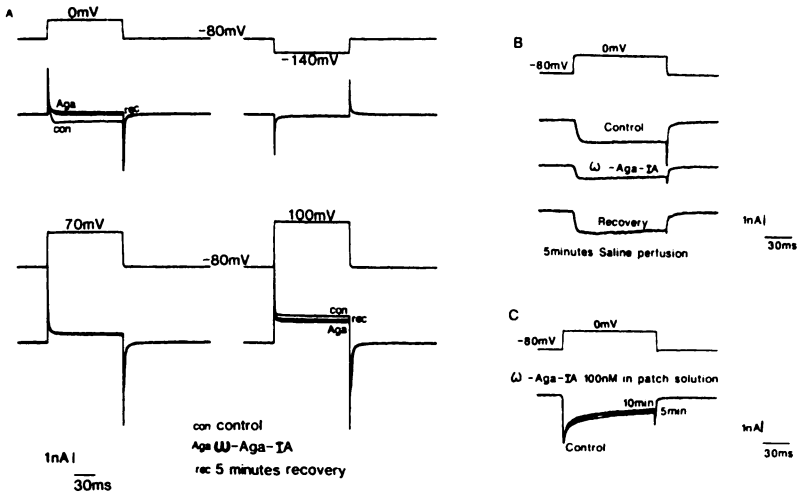


Fig. 2. Block of I_{Ba} by ω-Aga-IA and its reversibility in DRG neurons. A, The action of 10 nM ω-Aga-IA, applied for 4 min, on I_{Ba} (V_c = 0 mV), leak current (V_c = −140 mV), the current activated at the null potential (V_c = 70 mV), and the outward Ca^{2+} channel current (V_c = 100 mV). Three currents are superimposed in each of the panels, showing inhibition by ω-Aga-IA followed by 5-min partial recovery of I_{Ba} and the outward Ca^{2+} channel current. The leak current and current at the null potential at V_c = −140 mV and 70 mV are unchanged by ω-Aga-IA. The null potential is the potential at which no net inward or outward current is activated; however, it is not a true reversal potential. Recovery was dependent on diffusion away from the cell and was slight (<20%). B, Partial inhibition of I_{Ba} by a 1-min application of ω-Aga-IA (1 nM); recovery was facilitated by perfusion with fresh saline for 5 min. More impressive recovery following deeper block by ω-Aga-IA was not achieved in this study. C, ω-Aga-IA (100 nM) applied intracellularly via the patch solution did not markedly inhibit the I_{Ba} activated at 0 mV. *Traces* show I_{Ba} activated within 30 sec of entering the whole-cell recording configuration (control) and 5 and 10 min later.

application for 3 min, the recovery of I_{Ba} was only slight (<20%) (Fig. 2A). The poor recovery observed suggests that the toxin has a slow dissociation rate. Better recovery was observed when the cells were perfused with fresh saline for 5 min following a shorter (1-min) application of 1 nM ω-Aga-IA (Fig. 2B, Table 1).

ω-Aga-IA had little effect when applied intracellularly. Inclusion of 100 nM ω-Aga-IA in the patch solution had only modest effects on the amplitude and kinetics of I_{Ba}. These were probably due to leakage from the patch pipette before contact with the cell. Little change in I_{Ba} was observed when company currents activated 10 sec after entry into the whole-cell recording configuration with those activated 10 min later ($n = 7$) (Fig. 2C).

At $V_H = -30$ mV, much of the high threshold I_{Ba} is inactivated and the current activated at $V_c = 0$ mV is predominantly, but not exclusively, due to L-type Ca^{2+} channel activity. The sustained I_{Ba} activated from $V_H = -30$ mV by 30-mV voltage step commands was 1.47 ± 0.33 nA ($n = 5$) in amplitude and was reduced by 10 nM ω-Aga-IA by 88 ± 4%, to 0.20 ± 0.07 nA. The steady state inactivation curve for high threshold I_{Ba} ($V_c = 0$ mV) was shifted to more hyperpolarized levels in three of three cells by ω-Aga-IA (10 nM). The voltage for 50% steady state inactivation of I_{Ba} ($V_{0.5}$) was −41 ± 10 mV under control conditions and −52 ± 8 mV ($n = 3$) in the presence of ω-Aga-IA.

In contrast to the action of ω-Aga-IA, ω-CgTx irreversibly inhibited I_{Ba} recorded from DRG neurons. The high threshold I_{Ba} activated at $V_c = 0$ mV was inhibited by 1 μM ω-CgTx by 72 ± 4% and 74 ± 5% ($n = 14$), for the current measured at its peak and at the end of a 100-msec voltage step command, respectively. A similar degree of block of 84 ± 8% ($n = 6$) was seen when 1 μM ω-CgTx was applied to cells held at $V_H = -30$ mV and I_{Ba} was activated at $V_c = 0$ mV. We and others have previously reported the lack of an inhibitory action of ω-CgTx (1 μM) on the low threshold T-type current (6, 20), although higher concentrations produce some inhibition.

Effect of ω-Aga-IA on DRG Ca^{2+} currents. The characteristics of inhibition of voltage-activated Ca^{2+} currents (I_{Ca}) by ω-Aga-IA were different from those described for I_{Ba}. ω-Aga-IA reduced the sustained I_{Ca} measured at the end of the 100-msec voltage step command to a much greater extent than the peak current (Figs. 3, 4, and 5 and Table 2). Under control conditions, the mean amplitude of I_{Ca} (5 mM Ca^{2+} present in the recording medium) was 3.0 ± 0.4 nA and the current inactivated to 1.6 ± 0.2 nA ($n = 6$). Application of 10 nM ω-Aga-IA markedly reduced I_{Ca}; after a 3-min application, the peak current was inhibited by 57% to 1.3 ± 0.2 nA, whereas the current measured at the end of the voltage step command was inhibited by 94% to 0.07 ± 0.03 nA (Fig. 3A, Table 2). This

TABLE 1

Reversibility of the effect of 1 nM ω-Aga-IA on I_{Ba} measured at the peak and at the end of the voltage step command to $V_c = 0$ mV

	n	I_{Ba}	
		Peak	End
		nA	
Control	4	3.6 ± 1.1	2.5 ± 0.5
ω-Aga-IA, 1 nM	4	1.9 ± 0.5	1.36 ± 0.5
Recovery after 5-min perfusion with fresh saline	4	3.1 ± 0.5	2.1 ± 0.3

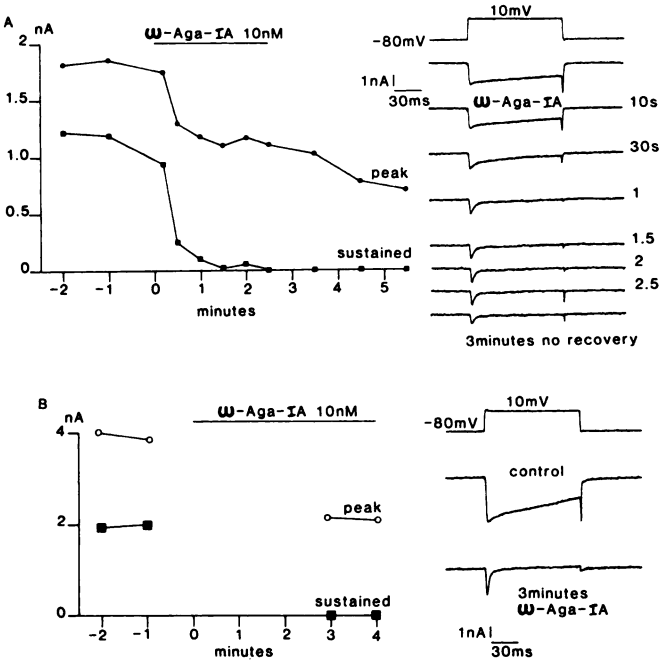


Fig. 3. Action of ω-Aga-IA on I_{Ca} recorded from DRG neurons. A, Time course of decline, induced by ω-Aga-IA, of peak (●) and sustained (■) I_{Ca} activated from $V_H = -80$ mV by 100-msec depolarizing voltage step commands to $V_c = 10$ mV, induced by 2.5-min low pressure ejection of 10 mM ω-Aga-IA. During the application of ω-Aga-IA, I_{Ca} was activated every 30 sec. The current measured at the end of the 100-msec voltage step command (sustained) is inhibited by ω-Aga-IA to a greater extent than the peak current. No recovery was observed for the 3 min following termination of ω-Aga-IA application. B, Time course of decline of I_{Ca} [peak (○) and sustained (■)] induced by ω-Aga-IA. In this experiment, I_{Ca} was only activated after a 3-min application of the toxin. The sustained current (■) was abolished by ω-Aga-IA; this was not dependent on repeated activation of I_{Ca}.

rapid residual I_{Ca} was also observed following inhibition by ω-Aga-IA applied to freshly replated DRG neurons, which had no processes.[1] The inhibitory actions of ω-Aga-IA on I_{Ca} were concentration dependent (Table 2); this is clear even though each concentration of ω-Aga-IA was applied to a different cell. We were unable to detect any significant change in I_{Ca} after a 3-min application of 0.01 nM ω-Aga-IA.

The virtual abolition of the sustained I_{Ca} induced by 10 nM ω-Aga-IA was not dependent on test currents being activated during the onset of block. The inhibition was apparent when a single I_{Ca} was activated after a 3-min application of 10 nM ω-Aga-IA and was observed to be inhibited by 100 and 97% ($n = 2$) (Fig. 3B).

The more marked ω-Aga-IA-induced inhibition of I_{Ca} measured at the end of the voltage step command was observed for both inward and outward currents. As observed for inward I_{Ca}, ω-Aga-IA inhibited the sustained outward I_{Ca} to a greater extent than the fast transient component (Fig. 4A). To test for the involvement of T-type channels in the fast inward transient current, which persisted following ω-Aga-IA treatment, two types of experiment were performed. Firstly, 1-octanol (10 μM) was applied to cells after ω-Aga-IA had reduced I_{Ca} to a fast transient current. 1-Octanol has previously been shown to be an inhibitor of the T-type current in some cells, including DRG neurons (6). Application of 10 μM 1-octanol had no significant effect on the residual fast transient current recorded in the continued presence of ω-Aga-IA (Fig. 4B). In the second type

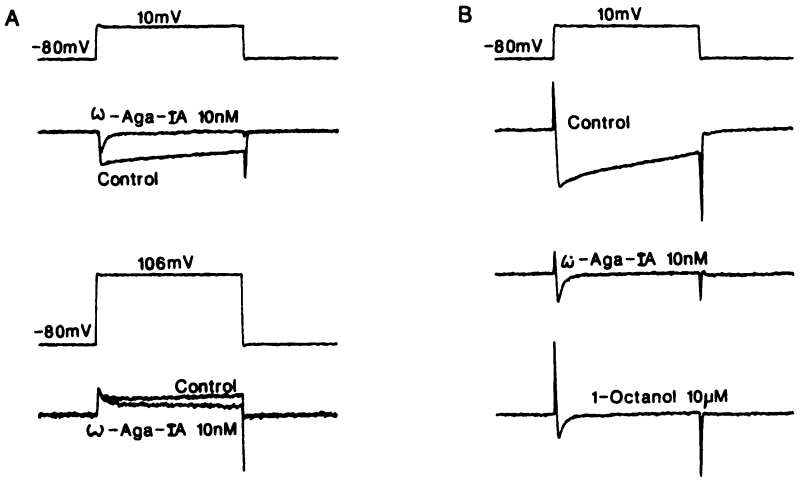


Fig. 4. Inhibition of I_{Ca} by ω-Aga-IA in DRG neurons. A, Both inward I_{Ca} and outward Ca^{2+} channel currents, activated at V_c = 10 mV and 106 mV, respectively, were inhibited by a 5-min application of 10 nM ω-Aga-IA, recorded in the same cell. Preferential inhibition of current measured at the end of the 100-msec voltage step command is observed in both I_{Ca} and the outward current. A modest increase in outward current can be observed during a 100-msec step and may reflect the absence of Ca^{2+}-induced inactivation and delayed opening of some channels, at this very depolarized potential. B, High threshold I_{Ca} activated at V_c = 10 mV was inhibited by a 3-min application of ω-Aga-IA. The residual fast transient current recorded in the presence of ω-Aga-IA was not markedly affected by a 3-min application of the T-type current inhibitor 1-octanol (10 μM).

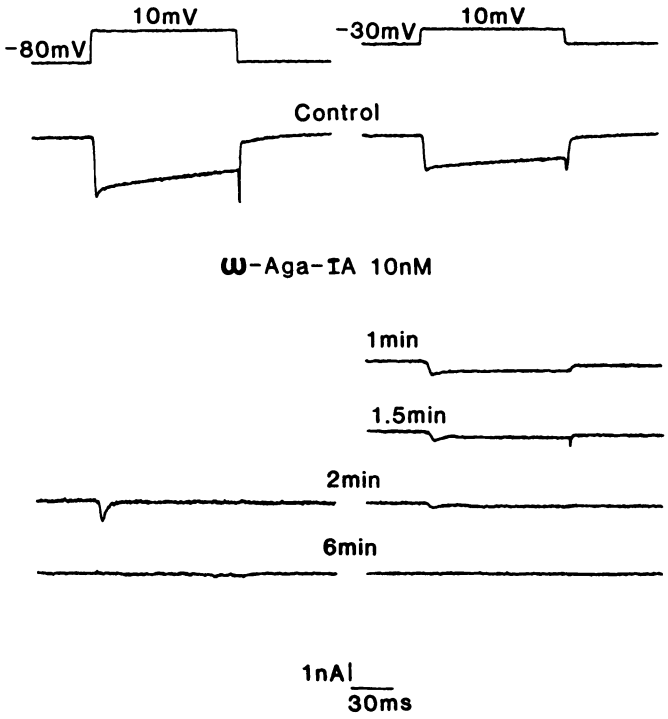


Fig. 5. Inhibition induced by ω-Aga-IA of I_{Ca} activated from two holding potentials. *Traces* show the maximum I_{Ca} activated at V_c = 10 mV from V_H = −80 mV and −30 mV under control conditions and during application of 10 nM ω-Aga-IA. After 2-min and 6-min applications of ω-Aga-IA, I_{Ca} was activated from both V_H = −80 mV (*left*) and V_H = −30 mV (*right*). After a 2-min application of ω-Aga-IA, the fast transient I_{Ca} activated from V_H = −30 mV appears to be reduced to a greater extent, suggesting that most of this component of I_{Ca} is inactivated or that part of the ω-Aga-IA action is voltage dependent. In this particular cell, 6-min application of ω-Aga-IA abolished I_{Ca} completely.

of experiment, DRG neurons were held at V_H = −30 mV, which completely inactivates T-type currents. ω-Aga-IA inhibited the residual I_{Ca} activated by 100-msec step depolarizations to V_c = 0 mV and, although the current became more transient in nature during the onset of block, this effect was less apparent than when cells were held at V_H = −80 mV (Fig. 5). In the experiment illustrated in Fig. 5, after a 6-min application of 10 nM ω-Aga-IA I_{Ca} was completely inhibited. These data suggest that T-type Ca^{2+} channel activity probably does not underlie the fast transient current.

TABLE 2

Dose-dependent action of ω-Aga-IA (applied for 3 min) on I_{Ca} recorded from DRG neurons

ω-Aga-Ia	n	Inhibition of I_{Ca}	
		Peak	End[a]
nM		%	
10	6	57 ± 4	94 ± 3
1	6	50 ± 14	68 ± 11
0.1	5	31 ± 10	54 ± 13

[a] At the end of the voltage step command.

Effect of ω-Aga-IA on Na^+ currents recorded from DRG neurons. Experiments were performed to study whether ω-Aga-IA had any effect on sodium currents (I_{Na}), to investigate the possibility that the residual fast transient current mentioned in the previous section was carried by sodium. In four experiments, ω-Aga-IA (10 nM) had no effect on the voltage-activated I_{Na} (V_H = −80 mV; V_c = −20 mV) recorded from DRG neurons (Fig. 6A). Application of TTX (2 μM) inhibited the I_{Na}, leaving the residual I_{Ca} present at V_c = −20 mV intact (Fig. 6B).

Actions of ω-Aga-IA and ω-CgTx on the dihydropyridine-sensitive component of I_{Ba} recorded from DRG neurons. The 1,4-dihydropyridine (+)-202-791 acts as a Ca^{2+} channel agonist and enhances the L-type component of the high threshold current. Application of (+)-202-791 (5 μM) produced an enhancement of I_{Ba}, increasing the peak current from 2.7 ± 0.3 nA to 3.5 ± 0.2 nA (n = 13). The mean percentage of increase in I_{Ba} induced by (+)-202-791 was 40 ± 14% and 39 ± 10%, for the peak current and the current measured at the end of the voltage step command, respectively. All cells studied were sensitive to (+)-202-791 and were also markedly inhibited (78 ± 7%; n = 6) by ω-Aga-IA (10 nM). In two of six cells, the I_{Ba} shown to be sensitive to (+)-202-791 was abolished by subsequent application of ω-Aga-IA (Fig. 7A). In contrast, ω-CgTx (1 μM) partially inhibited I_{Ba} (50 ± 7%, n = 5) and subsequent application of (+)-202-791 (5 μM) enhanced in a fully reversible manner the residual current, by 35 ± 23% and 24 ± 11% (n = 5) for the peak current and the current measured at the end of the voltage step command, respectively (Fig. 7B). There was no significant difference between the mean percentages of increase induced by (+)-202-791 in control I_{Ba} and the residual I_{Ba} activated after ω-CgTx application.

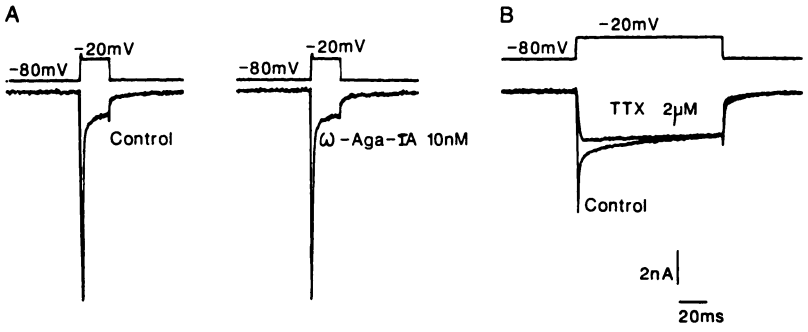


Fig. 6. Action of ω-Aga-IA on I_{Na} recorded from DRG neurons. A, Traces of I_{Na} recorded in the absence of TTX but in the presence of K^+ channel blockers. I_{Na} was activated from V_H = −80 mV by 20-msec depolarizing voltage step commands to V_c = −20 mV (some overshoot in the voltage was observed, due to the large amplitude of I_{Na}). Neither the fast transient nor the more slowly decaying component of I_{Na} was inhibited by a 3-min application of ω-Aga-IA (10 nM). B, An inward current (Na^+ and Ba^+) activated from V_H = −80 mV by a 100-msec voltage step command to V_c = −20 mV. Application of 2 μM TTX inhibited the I_{Na} and revealed I_{Ba}. These data showed that the fast transient and the more slowly decaying components of I_{Na} were sensitive to TTX.

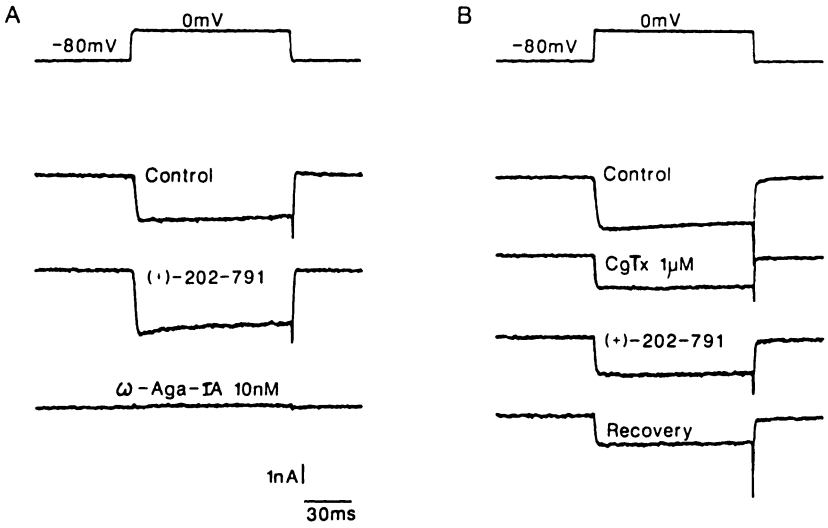


Fig. 7. Actions of ω-Aga-IA and ω-CgTx on the dihydropyridine-sensitive component of I_{Ba} in DRG neurons. A, Control I_{Ba} activated at V_c = 0 mV by 100-msec depolarizing voltage step commands. The dihydropyridine-sensitive component of I_{Ba} was enhanced by the agonist (+)-202-791 (5 μM) applied under conditions of low illumination. In the same cell, ω-Aga-IA (10 nM) was then applied and in this case completely abolished I_{Ba} over 5 min. B, I_{Ba} partially inhibited by 1 μM ω-CgTx applied for 5 min. The ω-CgTx-insensitive component of I_{Ba} was then observed to be enhanced reversibly by (+)-202-791 (5 μM).

Discussion

ω-Aga-IA, a toxin purified from the venom of the funnel web spider, potently inhibited high voltage-activated Ca^{2+} channel currents and had a less pronounced action on low threshold T-type currents. These findings may indicate structural differences in Ca^{2+} channels that underlie high and low threshold currents. Alternatively, each Ca^{2+} channel subtype may interact differently with other membrane constituents to which the toxin may bind. It is, however, clear that ω-Aga-IA binds to a site on the extracellular side of the cell. Evidence also suggests that ω-Aga-IA is a more potent inhibitor of neuronal Ca^{2+} channels than smooth muscle Ca^{2+} channels. Preliminary data using the rabbit ear artery indicate that ω-Aga-IA inhibits Ca^{2+} channels in this preparation with an IC_{50} of about 500 nM.[3]

The outward Ca^{2+} channel current was reduced by ω-Aga-IA but by a modest amount, compared with the inhibition of the high voltage-activated inward Ca^{2+} channel current in the same cell. This apparent difference in sensitivity was observed despite the fact that the kinetics of the residual outward current reflected those of the inward I_{Ca} (Fig. 4A). It may be possible to account for this discrepancy by the fact that the outward Ca^{2+} channel current may be contaminated by varying proportions of nonspecific cation channel activity (21). Our previous study showed that a substantial part of the net outward current under these conditions was blocked by Cd^{2+} or ω-CgTx (19). Furthermore, the outward current is also regulated by the γ-aminobutyric $acid_B$ receptor agonist (−)-baclofen and by GTP-γ-S, which activates GTP-binding proteins that functionally couple receptors to voltage-activated Ca^{2+} channels. However, inward and outward Ca^{2+} channel currents do show different steady state inactivation properties, with the latter current being less sensitive to depolarization (19). It is also possible that ω-Aga-IA is less efficient at blocking outward movement of monovalent cations than inward movement of divalent cations through Ca^{2+} channels. The action of ω-Aga-IA on the outward current supports the hypothesis that some of this current is carried through Ca^{2+} channels and that the inhibitory action of the toxin is not due to a shift in the voltage dependence of Ca^{2+} channels.

Complete inhibition of the dihydropyridine (+)-202-791-enhanced I_{Ba} by ω-Aga-IA clearly indicates that the toxin blocks neuronal L- as well as N-type currents. Furthermore, all cells studied were sensitive to ω-CgTx, which has been found in some previous studies to differentiate N channels from L channels (5, 22). Thus, we conclude that ω-Aga-IA does not distinguish between N- and L-type components of the high voltage-activated I_{Ba}.

A number of possibilities may account for the clear differences in the kinetics of inactivation of the residual I_{Ca} and I_{Ba} following application of ω-Aga-IA.

(a) The permeant ion affects the characteristics of inhibition. The mechanism of action of ω-Aga-IA could involve modulation of the Ca^{2+}-dependent inactivation process (23) or an effect on the cation binding sites within the channel. The size of the peptide toxin and its lack of activity when applied intracellularly suggest that toxin interactions result in allosteric changes

[3] A. Hughes and S. Hering, personal communication.

to the channel. Such allosteric changes could, therefore, result in enhancement in the efficiency of Ca^{2+} channel inactivation mediated by Ca^{2+} or a differential effect on ions (Ba^{2+}, Ca^{2+}, Cs^+, or K^+) passing through the Ca^{2+} channels.

(b) The fast transient residual I_{Ca} observed following application of ω-Aga-IA could be an unmasked T-type current. This possibility is unlikely, because it would be equally apparent when Ba^{2+} or Ca^{2+} was the charge carrier and T currents are not seen in every cell, whereas the fast transient residual I_{Ca} was. Additionally, the fast transient residual I_{Ca} was activated at large depolarized clamp potentials (V_c = 0 to 10 mV), and it was insensitive to 1-octanol (1 μM), which abolishes T-type currents in DRG neurons.

(c) It is also possible that the fast transient residual I_{Ca} recorded in the presence of ω-Aga-IA is a TTX-insensitive I_{Na} (24). This explanation is unlikely but cannot be completely ruled out. Evidence for excluding this possibility includes the observation that the residual transient current is gradually reduced and in some cases abolished by the toxin. In contrast, I_{Na} was unaffected by the toxin. Furthermore, the residual transient current was not seen when Ba^{2+} was the charge carrier, which would be unexpected if this current was a Na^+ current.

(d) A fourth possibility is that the residual transient I_{Ca} is located in distal neurites. This explanation is unlikely, because the responses can be reproduced in freshly replated DRG neurons, which do not have any processes. Furthermore, in our experimental system the processes were also well bathed in ω-Aga-IA.

In a comparison of the actions of ω-Aga-IA and ω-CgTx on voltage-activated Ca^{2+} channel currents in DRG cells, a number of differences are apparent. (a) ω-Aga-IA has a partially reversible action, whereas ω-CgTx inhibition is irreversible. (b) ω-Aga-IA is several orders of magnitude more potent than ω-CgTx, in a comparison of currents activated following 5-min applications of the toxins. (c) Over the first 5 min of application, ω-Aga-IA blocks I_{Ca} measured at the end of a 100-msec voltage step command to a greater extent than the peak current. If applied for longer periods, ω-Aga-IA completely blocked the transient component in some cells. No such transient current is observed when ω-CgTx is applied (8). (d) ω-Aga-IA blocks all components of the high voltage-activated Ca^{2+} channel current, whereas it has previously been suggested that ω-CgTx preferentially inhibits the N-type component, which is insensitive to dihydropyridines (5), although in the original studies it was found to inhibit both N and L currents (8). In the present experiments, the currents activated before and after ω-CgTx application were equally sensitive to dihydropyridine agonist. Although the amount of enhancement due to (+)-202-791 was variable between cells, equal sensitivity of control and residual currents to this dihydropyridine following application of ω-CgTx led us to conclude that in our hands this toxin inhibits both N- and L-type currents in DRG neurons. It is also worth noting that, in some DRG neurons, ω-CgTx completely abolishes the high voltage-activated Ca^{2+} channel current (6, 8, 19). This may be due to variation between cells in the expression of different Ca^{2+} channels as well as varying specificity of ω-CgTx from different sources (purified or synthesized). Binding studies suggest that the two toxins, ω-Aga-IA and ω-CgTx, have two distinct sites of action (11).

We conclude that ω-Aga-IA is a potent inhibitor of neuronal voltage-activated Ca^{2+} channel currents recorded from cultured DRG and cerebellar neurons and may prove useful in the purification of Ca^{2+} channels and investigation of their physiology. A polyamine toxin (funnel web spider toxin) has also been isolated from funnel web spider venom and has been found to inhibit a novel Ca^{2+} channel in cerebellar Purkinje neurons (25). We are presently investigating whether other peptide toxins isolated from the funnel web spider venom will prove to be selective in their action on different subtypes of Ca^{2+} channels.

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