A Hot Spot for the Interaction of Gating Modifier Toxins with Voltage-dependent Ion Channels

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ABSTRACT The gating modifier toxins are a large family of protein toxins that modify either activation or inactivation of voltage-gated ion channels. ω-Aga-IVA is a gating modifier toxin from spider venom that inhibits voltage-gated Ca^{2+} channels by shifting activation to more depolarized voltages. We identified two Glu residues near the COOH-terminal edge of S3 in the α_{1A} Ca^{2+} channel (one in repeat I and the other in repeat IV) that align with Glu residues previously implicated in forming the binding sites for gating modifier toxins on K^+ and Na^+ channels. We found that mutation of the Glu residue in repeat I of the Ca^{2+} channel had no significant effect on inhibition by ω-Aga-IVA, whereas the equivalent mutation of the Glu in repeat IV disrupted inhibition by the toxin. These results suggest that the COOH-terminal end of S3 within repeat IV contributes to forming a receptor for ω-Aga-IVA. The strong predictive value of previous mapping studies for K^+ and Na^+ channel toxins argues for a conserved binding motif for gating modifier toxins within the voltage-sensing domains of voltage-gated ion channels.

KEY WORDS: calcium channels • mutagenesis • agatoxin • voltage-sensing domain

INTRODUCTION

The voltage-gated K^+, Na^+, and Ca^{2+} channels are a large family of membrane proteins that share many structural features (Armstrong and Hille, 1998; Catterall, 2000). Voltage-gated K^+ channels are formal tetramers with each α subunit containing six (S1–S6) transmembrane segments, all of which seem to be membrane-spanning α-helices (Doyle et al., 1998; MacKinnon et al., 1998; Monks et al., 1999; Hong and Miller, 2000; Li-Smerin et al., 2000). A central pore domain, which contains the ion conduction pathway, is formed by the tetrameric arrangement of the S5–S6 regions (Doyle et al., 1998; MacKinnon et al., 1998). Four equivalent voltage-sensing domains, each composed of the first four segments (S1–S4), surround the central pore domain (for review see Li-Smerin et al., 2000). In voltage-gated Na^+ and Ca^{2+} channels, the general architecture is similar to K^+ channels, with the difference being that each α subunit contains four repeating psuedosubunits, each resembling a single K^+ channel α subunit (Catterall, 1996; Armstrong and Hille, 1998; Catterall, 2000). Although the four internal repeats in voltage-gated Ca^{2+} and Na^+ channel α subunits are homologous, they are not identical.

Venomous animals produce an almost limitless array of different protein toxins, many of which interact with voltage-gated ion channels. Some protein toxins bind to the outer vestibule of the ion conduction pore, and they are thought to inhibit the channel by physically occluding ion conduction (Hille, 1992; Miller, 1995; Catterall, 1996). Other types of protein toxins interact with very different regions of voltage-gated ion channels and perturb channel function by a distinct mechanism. These toxins are known as gating modifiers because they perturb the energetics of either activation or inactivation gating. The protein toxins that are established gating modifiers include the following: the α- and β-scorpion toxins, sea anemone toxins, and δ-conotoxins for Na^+ channels (Cahalan, 1975; Catterall, 1979; Wang and Strichartz, 1983; Meves et al., 1986; Strichartz and Wang, 1986; Gonoi and Hille, 1987; Norton, 1991; Fainzilber et al., 1994, 1995; Shon et al., 1994; Hanck and Sheets, 1995; Rogers et al., 1996; Cestele et al., 1998); Hanatoxin for K^+ channels (Swartz and MacKinnon, 1997a,b; Li-Smerin and Swartz, 1998, 2000; Swartz, 1999); and ω-Aga-IVA, Grammotoxin, and Kurtoxin for Ca^{2+} channels (Mintz et al., 1992a,b; Lampe et al., 1993; McDonough et al., 1997a,b; Chuang et al., 1998).

Suppose that gating modifier toxins interact exclusively with the voltage-sensing domains, as seems to be

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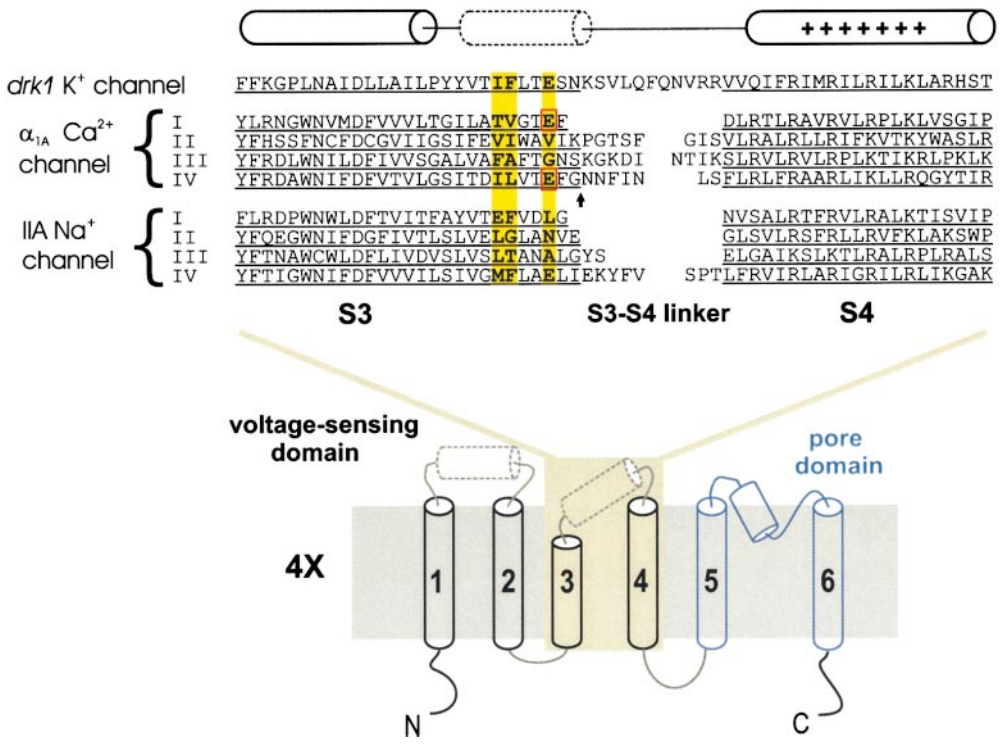

FIGURE 1. Sequence comparison between voltage-gated ion channels in the gating modifier toxin binding region. Membrane folding model for a K^+ channel α subunit and sequence alignment between K^+, Ca^{2+}, and Na^+ channels in S3–S4. Cylinders demarcate the approximate positions of α-helices based on scanning mutagenesis in both *Shaker* and *drk* K^+ channels (Monks et al., 1999; Hong and Miller, 2000; Li-Smerin et al., 2000). Underlined region of sequence marks approximate transmembrane segments identified by hydrophobicity analysis. The three positions highlighted in yellow correspond to positions in the *drk1* K^+ channel (Ile273, Phe274, and Glu277) where mutations alter Hanatoxin affinity (Swartz and MacKinnon, 1997b; Li-Smerin and Swartz, 1998, 2000). Mutation of Glu1613 in repeat IV of the Na^+ channel (highlighted yellow) decreases the binding affinity of α-scorpion and sea anemone toxins (Rogers et al., 1996). In the α_{1A} Ca^{2+} channel, two repeats contain Glu residues (Glu188 and Glu1658; marked by yellow highlighting and the red box) at positions corresponding to Glu277 in the *drk1* K^+ channel and Glu 1613 in the brain IIA Na^+ channel. The black arrow under the sequence for repeat IV in the α_{1A} Ca^{2+} channel marks the position where insertion of Asn and Pro has been reported to decrease ω-Aga-IVA affinity (Bourinet et al., 1999; Hans et al., 1999).

the case for Hanatoxin and voltage-gated K^+ channels (Li-Smerin and Swartz, 2000). Even in this case, there should be ample surface area on the extracellular surface of these domains to allow different gating modifier toxins to interact in unique ways with the voltage-sensing domains, possibly having few, if any, interacting pairs of residues in common. However, mapping studies show that homologous Glu residues in the COOH terminus of S3 in a K^+ channel, and the equivalent region of repeat IV in a Na^+ channel, are important determinants for Hanatoxin binding to the K^+ channel (Swartz and MacKinnon, 1997b; Li-Smerin and Swartz, 2000) and both α-scorpion and sea anemone toxin binding to the Na^+ channel (Rogers et al., 1996). This suggests that the K^+ channel toxin and the Na^+ channel toxins interact with very similar regions of their respective channels.

To further examine the generality of these results, we asked whether a gating modifier toxin for a voltage-gated Ca^{2+} channel would interact with the same region. The best studied gating modifier toxin for Ca^{2+} channels is ω-Aga-IVA (a protein toxin from spider venom) that inhibits P-type Ca^{2+} channel by shifting activation to more depolarized voltages (Mintz et al., 1992a,b; McDonough et al., 1997b). From the biophysical properties of the ω-Aga-IVA–Ca^{2+} channel interaction, we would predict that this toxin interacts with at least one of the voltage-sensing domains of the channel, which is similar to Hanatoxin and both α-scorpion and sea anemone toxins (Rogers et al., 1996; Swartz and MacKinnon, 1997a,b; Li-Smerin and Swartz, 2000). In this case, there could be anywhere from one to four ω-Aga-IVA receptors per channel because the Ca^{2+} channel contains four nonidentical voltage-sensing domains. The sequence alignment in Fig. 1 identifies two Glu residues in the α_{1A} Ca^{2+} channel (the cloned α subunit of native P-type channels), one in repeat I and the other in repeat IV, that correspond to the Glu residues in K^+ and Na^+ channels previously found to alter the binding of gating modifier toxins to these channels (Rogers et al., 1996; Swartz and MacKinnon, 1997b; Li-Smerin and Swartz, 2000). The Glu residue in repeat IV is of particular interest because insertion of Asn and Pro at the nearby position marked by the black arrow in Fig. 1 has been reported to decrease ω-Aga-IVA affinity by ~10-fold (Bourinet et al., 1999; Hans et al., 1999). We mutated each of these two Glu residues and assessed the effects on the interaction between ω-Aga-IVA and the Ca^{2+} channel. Our results support the general idea that gating modifier toxins interact with a similar region of the voltage-sensing domains in different voltage-gated ion channels.

MATERIALS AND METHODS

Molecular Biology

cDNAs encoding rabbit brain α_{1A} (BI-1) (Mori et al., 1991), rat brain β_{1b} (Pragnell et al., 1991), and rabbit skeletal muscle $\alpha_2\delta$ (Ellis et al., 1988) were provided by Lutz Birnbaumer (University of California, Los Angeles, Los Angeles, CA) in the pAGA2 vector (Wei et al., 1991). Point mutations were introduced into the α_{1A} cDNA by synthesizing mutant fragments using PCR, ligating into appropriately digested vectors, and sequencing using an automated DNA sequencer. α_{1A} and $\alpha_2\delta$ were linearized with XhoI, and β_{1b} was linearized with HindIII. All subunits were transcribed using T7 RNA polymerase.

Electrophysiology

Oocytes from *Xenopus laevis* frogs were removed surgically and incubated with agitation for 1.5 h in a solution containing: 82.5 mM NaCl, 2.5 mM KCl, 1 mM $MgCl_2$, 5 mM HEPES, and 2 mg/ml collagenase (Worthington Biochemical Corp.), pH 7.6, with NaOH. Defolliculated oocytes were injected with cRNA and incubated at 17°C in a solution containing: 96 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1.8 mM $CaCl_2$, 5 mM HEPES, and 50 μg/ml gentamicin (GIBCO BRL), pH 7.6, with NaOH for 1–7 d before electrophysiological recording. Approximately equal quantities (~10–100 ng) of cRNA for α_{1A}, β_{1b}, and $\alpha_2\delta$ subunits were injected into each oocyte. Oocyte membrane voltage was controlled using an oocyte clamp (model OC-725C; Warner Instruments). Data were filtered at 2 kHz (8-pole Bessel) and digitized at 10 kHz. Microelectrode resistances were 0.1–0.8 MΩ when filled with 3 M KCl. Oocytes were studied in 160–200-μl recording chambers perfused with a solution containing the following: 1 mM $BaOH_2$, 100 mM $NaCH_3SO_3$, and 10 mM HEPES, pH 7.6, with NaOH. Contamination of Ca^{2+} activated Cl^- current was minimized by recording in Cl^--free solution and recording Ba^{2+} currents usually <2 μA (mostly ~1 μA). 0.1 mg/ml^{-1} BSA was included in the extracellular recording solution to minimize adsorption of the toxin to the walls of the chamber and tubing. Agar salt bridges containing 1 M NaCl were used to connect the ground electrode pools and the recording chamber. All experiments were carried out at room temperature (~22–25°C). ω-Aga-IV was purchased from Calbiochem.

RESULTS

We studied the interaction of ω-Aga-IVA with the α_{1A} Ca^{2+} channel (Mori et al., 1991), the likely α subunit for P-type channels in cerebellar Purkinje neurons (Bourinet et al., 1999; Hans et al., 1999), expressed along with β_{1b} and $\alpha_2\delta$ accessory subunits in *Xenopus* oocytes. Fig. 2 shows the inhibitory effects of ω-Aga-IVA on the wild-type Ca^{2+} channel when using 1 mM Ba^{2+} as the charge carrier. At a concentration of 1 μM, ω-Aga-IVA produced nearly complete inhibition of Ba^{2+} currents elicited by test depolarizations to −15 mV. Even at this high toxin concentration, the rate of inhibition was rather slow; for the example shown in Fig. 2 B, the τ_{on} for onset of inhibition was ~50 s. Recovery of the current after removal of the toxin from the recording chamber was also slow, requiring that an oocyte be held in voltage-clamp for ~30 min to observe nearly complete recovery. A single exponential fit of the recovery in Fig. 2 B yielded a time constant of 370 s. The relationship between the fraction of uninhibited current and test voltage is shown in Fig. 2 C for several different toxin concentrations. With stronger test depolarizations, the fraction of uninhibited current increases. This fractional increase most likely represents the opening of toxin-bound channels at more depolarized voltages and possibly also the more rapid dissociation of the toxin from the open state (McDonough et al., 1997b). In the negative voltage range, the fraction of uninhibited current approaches a plateau value (Fig. 2 C). A similar plateau was observed at negative voltages for the fraction of uninhibited current with the interaction between Hanatoxin and a voltage-gated K^+ channel, where deactivation kinetics were found to be similar in the absence or presence of the toxin (Swartz and MacKinnon, 1997a). The results with Hanatoxin argue that tail current kinetics distinguish between channels opening with and without toxin molecules bound to them and imply that, at negative voltages, the fraction of uninhibited current approximates the fraction of unbound channels. For the present analysis of ω-Aga-IVA, we used an approach similar to that described for Hanatoxin, where the fraction of uninhibited current in the plateau phase at negative voltages is used to estimate the fraction of unbound channels at different toxin concentrations (Swartz and MacKinnon, 1997a). The fraction of uninhibited current at negative voltages (−25 to −15–mV range) is plotted against the concentration of ω-Aga-IVA in Fig. 2 D. An expression for 1:1 binding between toxin and channel was fit to the data with an equilibrium K_d of 160 nM.

We chose to mutate the two Glu residues in the α_{1A} Ca^{2+} channel that we identified in the sequence alignment in Fig. 1 to Lys (Glu188Lys and Glu1658Lys), a type of replacement that has particularly large effects on α-scorpion toxin binding to the Na^+ channel and Hanatoxin binding to the K^+ channel (Rogers et al., 1996; Li-Smerin and Swartz, 2000). The gating properties of Glu188Lys and Glu1658Lys are shown in Fig. 3 along with the wild-type channel. Both mutants produce only subtle changes in the voltage–activation relation, with ~10 mV rightward shifts in the conductance (G)–voltage (V) relation with little change in the slope of the voltage–activation relation. The effect of 1 μM ω-Aga-IVA on the wild-type and two mutant channels is shown in Fig. 4 (A and B). The current records in Fig. 4 A were elicited with very similar protocols using depolarizations to either –15 or −10 mV from a holding potential of −90 mV. 1 μM ω-Aga-IVA inhibited the repeat I mutant (Glu188Lys) and the wild-type channel to similar extents. In contrast, the same toxin concentration did not have a significant effect on channels containing the mutation in repeat IV (Glu1658Lys). Fig. 4 C shows

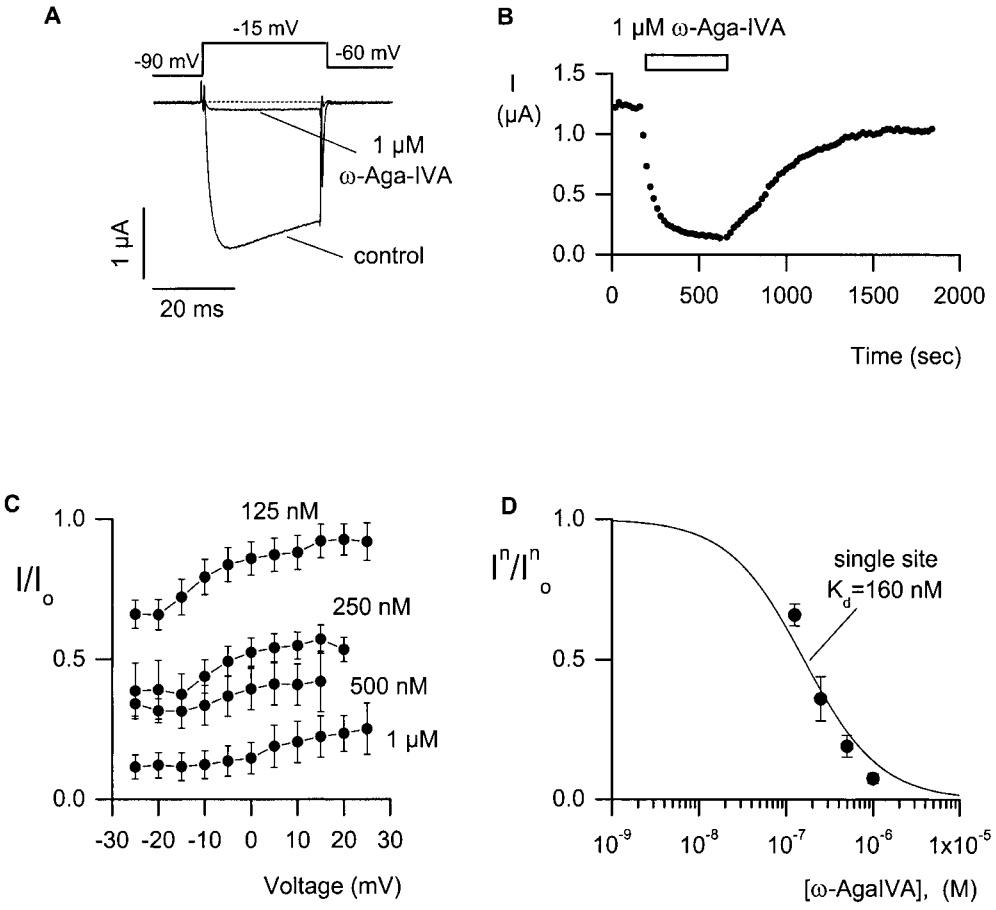

FIGURE 2. Inhibition of the α_{1A} Ca^{2+} channel by ω-Aga-IVA. (A) Inhibition of Ca^{2+} channel currents by ω-Aga-IVA. Current records elicited by depolarization in either the absence or presence of toxin. Charge carrier was 1 mM Ba^{2+}. Leak and capacitive currents have been isolated and subtracted using 100 μM Cd to block the Ca^{2+} channel. (B) Time course for onset and recovery for inhibition by ω-Aga-IVA. Steady state Ba^{2+} current elicited by depolarizations to −15 mV at 10-s intervals before and after application of the toxin. Inward Ba^{2+} currents were measured 10 ms after depolarization. (C) Fraction of uninhibited current elicited by various strength depolarizations. Steady state Ba^{2+} currents were elicited in the presence (I) or absence (I_0) of toxin using 30-ms depolarizations between −25 and +25 mV. Holding voltage was −90 mV in all cases. Data are means ± SEM for three oocytes at each toxin concentration. (D) Plot of fraction of uninhibited (I^n/I_0^n) current against toxin concentration. I^n/I_0^n is the fraction of uninhibited current in the plateau phase at negative voltages, typically –25 to –15 mV (see C). Smooth curve is a fit of $I^n/I_0^n = 1 - \{[\text{toxin}]/([\text{toxin}] + K_d)\}$ to the data, where K_d is the equilibrium dissociation constant for toxin binding to a single site on the channel. Data are from means ± SEM for between three and five oocytes at each toxin concentration.

the concentration dependence for inhibition of the three channels by ω-Aga-IVA. The fits of an equation for 1:1 stoichiometry between toxin and channel to the data for Glu188Lys and the wild-type channel yielded K_d values of 110 and 160 nM, respectively. In contrast, even at very high concentrations (4 μM), ω-Aga-IVA produced only minimal inhibition of the Glu1658Lys mutant channel. We estimate the dissociation constant for toxin binding to the repeat IV mutant to be >33 μM, corresponding to a change in toxin binding affinity of ~200-fold or greater. From these results, we conclude that ω-Aga-IVA binds to a receptor on the voltage-sensing domain within repeat IV of the Ca^{2+} channel. These results argue that equivalent Glu residues in the COOH-terminal part of S3 are important determinants of the gating modifier toxin receptors in voltage-gated K^+, Na^+, and Ca^{2+} channels.

DISCUSSION

We set out to find the location of the receptor for ω-Aga-IVA on a voltage-gated Ca^{2+} channel using the information already available for the location of gating modifier toxin receptors on Na^+ and K^+ channels. The alignment of K^+, Na^+, and Ca^{2+} channels from S3 through S4 identified two Glu residues in the α_{1A} Ca^{2+} channel (one in repeat I and the other in repeat IV) that are equivalent to Glu277 in the *drk1* K^+ channel and Glu1613 in the brain IIA Na^+ channel (Fig. 1). It has been previously reported that insertion of Asn and Pro (two positions COOH-terminal to Glu1658 in repeat IV of the α1A Ca^{2+} channel) decreases ω-Aga-IVA affinity, suggesting that a receptor for the toxin may be located nearby (Bourinet et al., 1999; Hans et al., 1999). We mutated each of these Glu residues (Glu188 and Glu1658) to Lys and studied the interaction between ω-Aga-IVA and the Ca^{2+} channel. Our results show that Glu1658Lys disrupts inhibition by ω-Aga-IVA, whereas Glu188Lys has no discernible effect (Fig. 4). This suggests that ω-Aga-IVA interacts with the voltage-sensing domain in repeat IV of the Ca^{2+} channel and that Glu1658 is a component of the toxin receptor. It is rather remarkable that the α_{1A} Ca^{2+} channel subunit contains >2,000 amino acids, yet it required only two

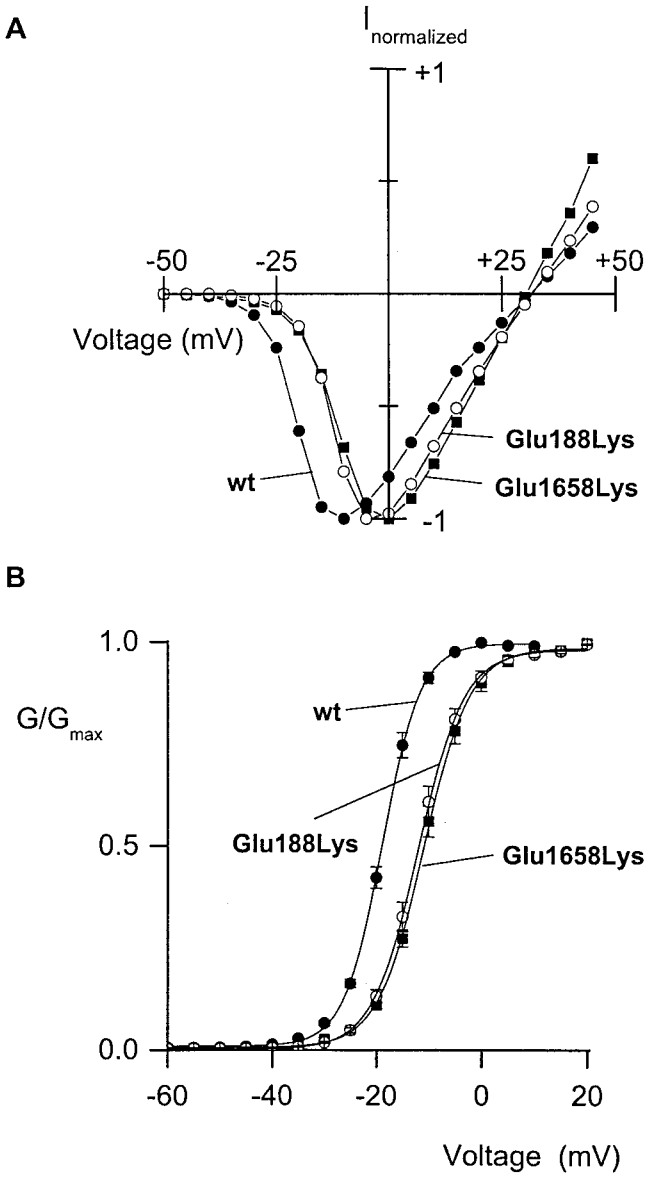

FIGURE 3. Gating properties of mutant α_{1A} Ca^{2+} channels. (A) Steady state current–voltage relations for wild-type and two mutant Ca^{2+} channels. Ba^{2+} currents were measured 10 ms after depolarization and have been normalized to the maximal inward current. Charge carrier was 1 mM Ba^{2+}. Leak currents were subtracted using 100 μM Cd. (B) Normalized conductance–voltage (G-V) relations for wild-type and two mutants channels. G was calculated according to $G = I_{Ba2+}/(V - V_{rev})$ with $V_{rev} = +29$ mV. Symbols are means ± SEM for four to five cells each. Smooth curves are single Boltzmann fits to the data with the following parameters: (wild-type) $z = 6.8$, $V_{50} = -18.9$ mV; (Glu188Lys) $z = 6.0$, $V_{50} = -11.9$ mV; and (Glu1658Lys) $z = 5.8$, $V_{50} = -10.9$ mV.

point mutations to locate the ω-Aga-IVA receptor. The predictive value of the K^+ and Na^+ channel toxin studies bolsters the idea that gating modifier toxins interact with a common region within the voltage-sensing domains of voltage-gated ion channels.

The idea of a canonical receptor for gating modifier toxins is further supported by several examples of gating modifier toxins that interact promiscuously with different types of voltage-gated ion channels. Hanatoxin, a K^+ channel inhibitor, is related in primary sequence to Grammotoxin, an inhibitor of voltage-gated Ca^{2+} channels, and both toxins can cross-react with the other's primary target (Li-Smerin and Swartz, 1998). In addition, at least in the K^+ channel, the binding of both toxins is altered by mutations in the COOH-terminal part of S3, arguing for a common binding site for the two toxins (Li-Smerin and Swartz, 1998). Another example of toxin promiscuity is seen with Kurtoxin, an α-scorpion toxin that was isolated in a screen for activity against T-type voltage–gated Ca^{2+} channels (Chuang et al., 1998). Kurtoxin inhibits the T-type Ca^{2+} channel by shifting activation to more depolarized voltages, reminiscent of both Hanatoxin and Grammotoxin. However, Kurtoxin also interacts with voltage-gated Na^+ channels and slows their inactivation, similar to other α-scorpion toxins to which it is related (Chuang et al., 1998). These results are consistent with the idea that gating modifier toxins interact with a similar region of voltage-gated channels and that this region adopts a well conserved 3-D structure.

Although the conserved Glu residues in voltage-gated K^+, Na^+, and Ca^{2+} channels probably mark the location of the gating modifier toxin binding sites, there are certainly other residues located at the toxin–channel interfaces that make significant energetic contributions. For the interaction of Hanatoxin with the *drk1* K^+ channel, at least two other residues (Ile273 and Phe274) contribute to form the toxin receptor (Li-Smerin and Swartz, 2000). It is interesting that both of these residues are well conserved in repeat IV of the Na^+ and Ca^{2+} channels (Fig. 1), although the effects of mutations at these positions on gating modifier toxins have not been reported. The 3-D structures of these gating modifier toxins (Widmer et al., 1989; Yu et al., 1993; Jablonsky et al., 1995; Kim et al., 1995; Monks et al., 1995; Reily et al., 1995; Takahashi et al., 2000) suggests that the toxin–channel interaction surfaces are probably somewhere between 20 × 20 Å and 30 × 30 Å in size, arguing that many channel residues will be buried in the toxin–channel complex. Whereas mutation of the Glu residue in repeat IV had dramatic effects, the equivalent mutation in repeat I was essentially silent. This is probably a manifestation of the fact that other residues in repeat I also determine the strength of the ω-AgaIVA–channel interaction and, thus, the toxin does not bind tightly to this repeat. An alternate interpretation would be possible if the voltage-sensitive conformational changes in repeat I are not strongly coupled to channel activation. In this case, repeat I might contain a fully competent ω-Aga-IVA binding

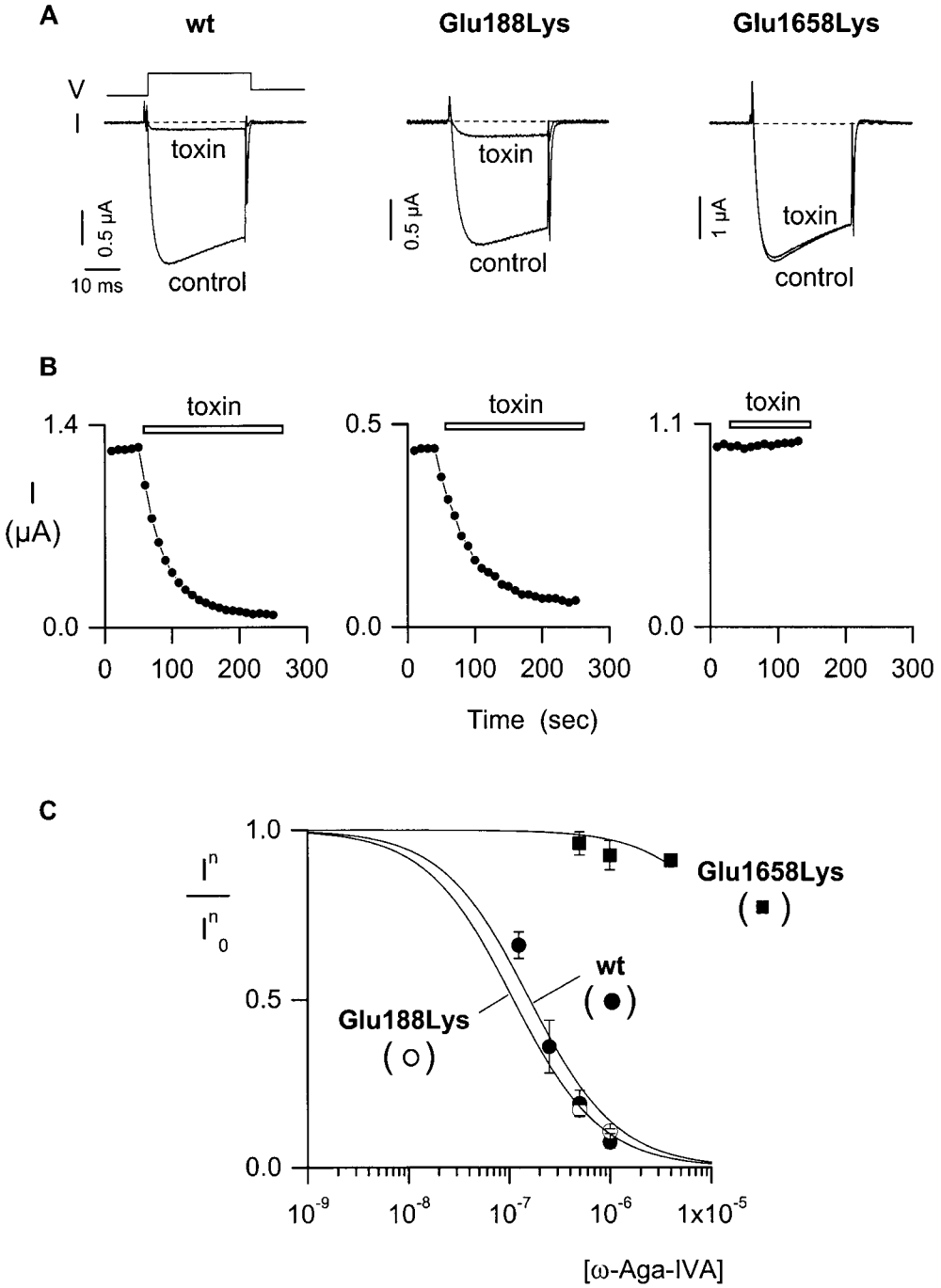

FIGURE 4. Altered interaction of ω-Aga-IVA with mutant Ca^{2+} channels. (A) Current records elicited by depolarization in the absence and presence of 1 μM ω-Aga-IVA. Depolarizations were elicited to −15 mV for wild type and to −10 mV for both Glu188Lys and Glu1658Lys. Holding voltage was −90 mV. Charge carrier was 1 mM Ba^{2+}. Leak and capacitive currents have been isolated and subtracted using 100 μM Cd. (B) Time course for effects of 1 μM ω-Aga-IVA. Steady state Ba^{2+} current elicited by depolarizations to either −15 mV (wild type) or −20 mV (both mutants) at 10-s intervals before and after application of toxin. Inward Ba^{2+} currents were measured 10 ms after depolarization. (Same cells as in A.) (C) Plot of fraction of uninhibited (I^n/I^n_0) current against toxin concentration for wild type, Glu188Lys, and Glu1658Lys. I^n/I^n_0 is the fraction of uninhibited current in the plateau phase at negative voltages, typically −25 to −5 mV (Fig. 2 C). Smooth curves are fits of $I^n/I^n_0 = 1 - \{[\text{toxin}]/([\text{toxin}] + K_d)\}$ to the data, where K_d is the equilibrium dissociation constant for single toxin binding sites. K_d values are as follows: 160 nM for wild-type; 110 nM for Glu188Lys; and 33 μM for Glu1658Lys. Data are means ± SEM for between three and eight oocytes at each toxin concentration. Data for wild type are from Fig. 2.

site, but our measurements simply don't allow us to detect occupancy.

What is the stoichiometry of the ω-AgaIVA–channel interaction? The concentration dependence for fractional inhibition of the channel by ω-AgaIVA (Fig. 2) deviated somewhat from that predicted for 1:1 stoichiometry. However, this deviation is most pronounced at low toxin concentrations where the binding kinetics are very slow (τ_{on} ~200 s at 125 nM toxin), possibly indicating a failure to reach equilibrium. We found that a single point mutation in repeat IV essentially disrupts inhibition by the toxin (Fig. 4). This result is consistent with the existence of a single receptor for the toxin on the Ca^{2+} channel, but does not rule out a number of more complex scenarios. It is possible that the toxin interacts with one or more of the three other repeats (I–III) of the Ca^{2+} channel. If these hypothetical interactions exist, they probably do not alter activation gating in isolation since the single point mutation in repeat IV (Glu1658Lys) essentially disrupts the inhibitory effects

of the toxin. Nevertheless, it is conceivable that toxin occupancy of repeats I, II, or III may be required for the inhibitory effect of the toxin on repeat IV.

Although the gating modifier toxins may interact with similar regions, it is interesting that some of these toxins have very different effects on channel gating. The toxins that bind to Na^+ channels slow inactivation, whereas the K^+ and Ca^{2+} channel toxins inhibit their channels by shifting activation to more depolarized voltages. In the case of Kurtoxin, the same molecule can elicit these two different effects: one when it binds to T-type Ca^{2+} channels, and the other when it binds to Na^+ channels (Chuang et al., 1998). One possibility is that the toxins exert different influences on the conformational rearrangements of the voltage-sensing domains in Na^+ channels versus K^+ and Ca^{2+} channels. An alternate explanation is that the effects of the toxins on the voltage-sensing domains are very similar, but it is the processes that the domains influence that are different. Thus, the voltage-sensing domain in repeat IV of the Na^+ channel might couple to channel inactivation as previously proposed (Sheets et al., 1999), whereas the equivalent repeat in the Ca^{2+} channel may couple to channel activation. The differential coupling of voltage-sensing domains to distinct gating processes seems less likely for K^+ channels given their fourfold symmetry. For all three channel types, the gating modifier toxins might perturb the conformational rearrangements in the voltage-sensing domains by stabilizing the resting conformation that predominates at negative voltages. This idea is supported by the observations that both the K^+ and Na^+ channel toxins inhibit gating currents in their respective channels (Nonner, 1979; Meves et al., 1987; Sheets and Hanck, 1995; Swartz, 1999) in spite of the fact that their effects on activation and inactivation are quite distinct.

It is interesting to consider why the gating modifier toxins target such a well-defined region within the voltage-sensing domains in different voltage-gated ion channels. In principle, there could be many distinct sites within the voltage-sensing domains from which different gating modifier toxins could alter the conformational rearrangements of the underlying protein. Yet, this seems not to be the case, at least not as a rule. One explanation might be that the conformational rearrangements occurring in the voltage-sensing domains are both spatially restricted and subtle. The gating modifier toxins might have evolved the ability to recognize a localized region where structural rearrangements can be most readily perturbed through protein–protein interactions.

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