

Pandinus imperator Scorpion Venom Blocks Voltage-gated Potassium Channels in GH₃ Cells

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ABSTRACT We examined the effects of *Pandinus imperator* scorpion venom on voltage-gated potassium channels in cultured clonal rat anterior pituitary cells (GH₃ cells) using the gigohm-seal voltage-clamp method in the whole-cell configuration. We found that *Pandinus* venom blocks the voltage-gated potassium channels of GH₃ cells in a voltage-dependent and dose-dependent manner. Crude venom in concentrations of 50–500 µg/ml produced 50–70% block of potassium currents measured at –20 mV, compared with 25–60% block measured at +50 mV. The venom both decreased the peak potassium current and shifted the voltage dependence of potassium current activation to more positive potentials. *Pandinus* venom affected potassium channel kinetics by slowing channel opening, speeding deactivation slightly, and increasing inactivation rates. Potassium currents in cells exposed to *Pandinus* venom did not recover control amplitudes or kinetics even after 20–40 min of washing with venom-free solution. The concentration dependence of crude venom block indicates that the toxins it contains are effective in the nanomolar range of concentrations. The effects of *Pandinus* venom were mimicked by zinc at concentrations ≤0.2 mM. Block of potassium current by zinc was voltage dependent and resembled *Pandinus* venom block, except that block by zinc was rapidly reversible. Since zinc is found in crude *Pandinus* venom, it could be important in the interaction of the venom with the potassium channel. We conclude that *Pandinus* venom contains toxins that bind tightly to voltage-dependent potassium channels in GH₃ cells. Because of its high affinity for voltage-gated potassium channels and its irreversibility, *Pandinus* venom may be useful in the isolation, mapping, and characterization of voltage-gated potassium channels.

INTRODUCTION

Specific toxins are valuable for categorizing, classifying, mapping, and purifying biological receptors of all types. This is especially true of ion channels, because it is difficult to assay channel activity by other than electrophysiological or tracer-flux methods, and these techniques are unsuited to dealing with populations of cells in situ or channel proteins outside of an intact membrane. Voltage-gated potassium

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channels comprise an important, widespread, and diverse category of ion channel for which relatively few specific ligands have been described. In this article, we examine the effects of a possible source of a specific ligand for voltage-gated potassium channels, venom from the scorpion, *Pandinus imperator*, on the voltage-gated potassium currents of cells from a mammalian pituitary cell line.

Previous studies have shown that *Pandinus* venom specifically and irreversibly blocks the voltage-gated potassium currents of myelinated nerve fibers (Pappone and Cahalan, 1987). In the present experiments, we examined the effects of *Pandinus* venom on potassium currents of voltage-clamped GH₃ cells. These experiments had two purposes. First, we wanted to examine the generality of *Pandinus* venom's effects on voltage-activated potassium channels by determining whether the non-neuronal potassium channels of GH₃ cells could also be blocked by the venom. Second, we wanted to find a preparation that could be easily patch-clamped and contained voltage-gated potassium currents susceptible to block by *Pandinus* venom. The gigohm-seal patch-clamp method (Hamill et al., 1981) is faster, easier, and more versatile than the Vaseline-gap technique used in our experiments on myelinated nerve fibers. Development of this more convenient assay system will be useful in future experiments attempting to purify the active component(s) from *Pandinus* venom. We find that the voltage-activated potassium currents of GH₃ cells are irreversibly blocked by *Pandinus* venom and that the effects of *Pandinus* venom on GH₃ cell potassium currents are similar to those described previously for axonal potassium channels. Preliminary reports of this work have been published in abstract form (Lucero and Pappone, 1987; Pappone and Lucero, 1987).

METHODS

Cells

GH₃ cells from the rat anterior pituitary tumor cell line were obtained from the American Type Culture Collection, Rockville, MD. The cells were grown in 70-mm plastic culture dishes in Dulbecco's modified Eagle's medium supplemented with 2.5% fetal bovine serum and 7.5% non-heat-inactivated horse serum. The cells were maintained at 37°C in a humidified atmosphere of 95% O₂ and 5% CO₂. The cells were subcultivated weekly and plated on acid-cleaned glass coverslips in 35-mm culture dishes. The medium was replaced every other day. Cells were used for patch-clamp experiments 3–15 d after subcultivation.

Solutions and Chemicals

Three basic external solutions were used in these experiments. Sodium Ringer consisted of 150 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 10 mM glucose, and 10 mM TMA-HEPES. Zero-potassium sodium Ringer contained 150 mM NaCl, 10 mM CaCl₂, 1 mM MgCl₂, and 5 mM TMA-HEPES. High-potassium Ringer contained 150 mM KCl, 1 mM MgCl₂, 5 mM TMA-HEPES, 100 μM TTX, and 10 or 2 mM CaCl₂. Zero-calcium, high-potassium Ringer was the same as the high-potassium Ringer, except that it contained 3 mM MgCl₂ and no CaCl₂. Internal solutions in the pipette were either KF-EGTA, which consisted of 140 mM KF, 1 mM CaCl₂, 2 mM MgCl₂, 11 mM K₂EGTA, and 10 mM TMA-HEPES, or KF-BAPTA, which consisted of 155 mM KF, 0.5 mM CaCl₂, 3 mM MgCl₂, 5.5 mM BAPTA, and 10 mM TMA-HEPES. Experiments examining sodium and calcium currents used an internal solution identical to the KF-EGTA, except that all potassium was replaced by cesium. All solutions had a pH of 7.4 and osmolarities of 290–

310 mosmol. Unless otherwise indicated, experiments were done with KF-EGTA as the internal solution and high-potassium Ringer with 10 mM calcium as the external solution. Lyophilized crude *Pandinus* venom was purchased from Latoxan, Rosans, France. A stock solution of 10 mg/ml venom in water was diluted with the appropriate Ringer solution to make the experimental test solutions. Tetraethylammonium (TEA) chloride (Sigma Chemical Co., St. Louis, MO, or Kodak Chemical Co., Rochester, NY) solution was made up by adding sufficient crystalline TEA to high-potassium Ringer solution to make the solution contain 10 mM TEA. This solution was then diluted with high-potassium Ringer for lower-concentration TEA solutions. The 4-aminopyridine (4-AP; Sigma Chemical Co.) solutions were made by dilutions of a 100-mM stock solution in water with appropriate amounts of high-potassium Ringer solution.

The effects of removing calcium from the bathing solution were tested by superfusing the cell with zero-calcium solution from a large (5–10 μm diam) pipette positioned $\sim 50 \mu\text{m}$ from the cell and connected to a Picospritzer (General Valve Corp., East Hanover, NJ) pressure-ejection system. The Picospritzer applied $\sim 10 \text{ lb/in.}^2$ of pressure to the pipette under control of the computer. Solution flow was started 200 ms before a voltage command pulse was delivered by the computer, and was maintained for the duration of the voltage pulse. These conditions were adequate to completely exchange the solution surrounding the cell during the voltage pulse, as evidenced by the fact that superfusion of a cell in sodium Ringer with high-potassium Ringer from the pipette resulted in current records that were identical to those recorded when the bulk solution was high-potassium Ringer.

Voltage-Clamp Recording

A sliver of coverslip with adherent GH₃ cells was placed in a glass-bottomed recording chamber that had a volume of $\sim 200 \mu\text{l}$. The cells were viewed at magnification of 400 using Nomarski interference contrast optics. Patch recording pipettes were constructed from Corning 7052 glass (Garner Glass, Claremont, CA) to have a resistance of 1–3 M Ω when filled with the internal solution. The pipettes were coated with Sylgard to reduce their capacitance. Membrane ionic currents were measured from cells voltage-clamped in the whole-cell configuration (Hamill et al., 1981) using a voltage-clamp amplifier (LM-EPC7, List-Medical, Darmstadt, Federal Republic of Germany). After formation of a gigaohm seal, the residual capacitance of the pipette was electronically nulled and a pulse of suction was applied with a large syringe to rupture the cell membrane beneath the pipette to achieve the whole-cell recording configuration. Passive properties of the cell membrane were determined from the average of 32 current responses to 10-mV hyperpolarizations of 10 ms duration. Cell capacitance was determined by integration of the transient current response, and membrane resistance was determined from the steady state current level at the end of the pulse. After measurement of the cell's passive properties, the current owing to the capacitance was electronically subtracted from subsequent records. For experiments examining the kinetics of membrane currents, 25–40% of the resistance in series with the cell membrane was compensated by positive feedback. All experiments were done at room temperature ($\sim 22^\circ\text{C}$).

Data Acquisition and Analysis

Voltage-clamp experiments were run on-line using a Cheshire Data Interface and an LSI 11/73 computer system (INDEC Systems, Sunnyvale, CA) to deliver command voltages and sample and store current data. The membrane potentials reported here have been corrected for the measured junction potential between the pipette and bath solutions. Membrane currents were low-pass-filtered using an eight-pole Bessel filter, sampled by a 12-bit A/D converter, and written into data files on the disk of the computer. Unless noted otherwise, the current records in these experiments were filtered with a cutoff of 440 Hz and sampled at

intervals of 458 μ s. Linear leak currents and residual uncompensated current through the membrane capacitance were measured using the P/4 procedure (C. M. Armstrong and Bezanilla, 1974), stored on the disk, and subsequently subtracted digitally before further analysis of the data.

Isolation of Voltage-gated Potassium Currents

GH₃ cells have membrane currents mediated by voltage-gated sodium channels (Dubinsky and Oxford, 1984; Fernandez et al., 1984; Matteson and Armstrong, 1984), voltage-gated calcium channels (Hagiwara and Ohmori, 1982; Dubinsky and Oxford, 1984, 1985; Matteson and Armstrong, 1984, 1986), voltage-gated potassium channels (Dubinsky and Oxford, 1984, 1985; Ritchie, 1987), and calcium-activated potassium channels (Dubinsky and Oxford, 1984, 1985; Ritchie, 1987). The amount of each current type present in any given GH₃ cell is highly

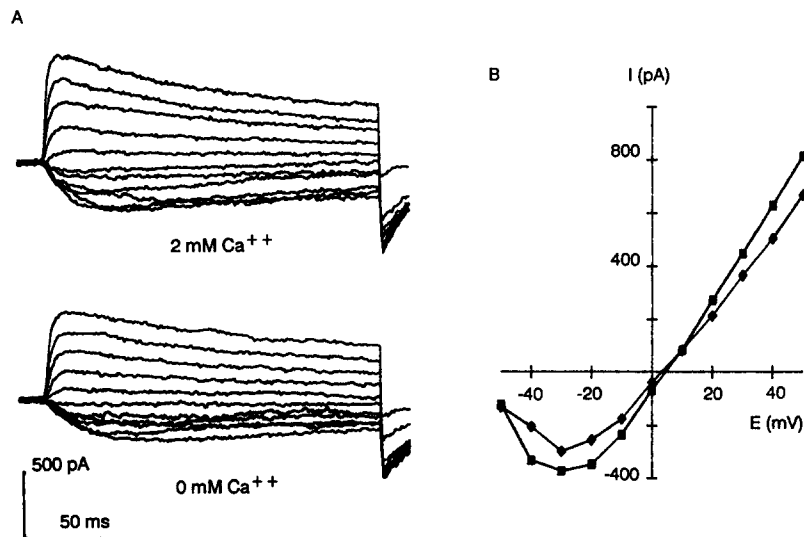


FIGURE 1. Effects of removing external calcium on potassium currents. (A) Membrane currents recorded in 2 mM calcium and 0 calcium, 3 mM magnesium external solutions. Membrane currents were recorded during 200-ms depolarizations from a holding potential of -80 mV to potentials from -50 to $+50$ mV in increments of 10 mV. The currents on the top were recorded in normal high-potassium solution containing 2 mM calcium. The currents on the bottom were recorded during superfusion of the cell with zero-calcium solution using the Picospritzer, as described in the Methods. (B) Peak current-voltage relation from the currents. Squares: peak current amplitude in 2 mM calcium. Diamonds: peak current amplitude in zero calcium, 3 mM magnesium. Cell PV42-2.

variable, and some current type may even be absent from a particular cell (Dubinsky and Oxford, 1984; Matteson and Armstrong, 1984).

In order to study the voltage-activated potassium currents in isolation, it was necessary to eliminate the other currents present in GH₃ cells. Sodium currents were blocked with tetrodotoxin (TTX). Since calcium channel blockers often affect potassium channels also (Cahalan et al., 1985), we chose not to attempt a pharmacological block of the channels. Rather, we took advantage of the variability in the amount of calcium current and the lability of the calcium channels in these cells (Hagiwara and Ohmori, 1982; Dubinsky and Oxford, 1984;

Matteson and Armstrong, 1986; Cohen and McCarthy, 1987; D. Armstrong and Eckert, 1987) to examine the voltage-activated potassium currents of GH₃ cells in isolation. We selected cells that showed little or no slow inward current initially, used fluoride solutions internally to increase the rate of disappearance of calcium channels (Carbone and Lux, 1985), and waited at least 10 min for the fast phase of calcium current washout to occur before collecting data (D. Armstrong and Eckert, 1987).

These procedures eliminated most or all of the calcium current and calcium-activated potassium current in our cells, as evidenced by the following results. Removal of all the external calcium resulted in a <10% change in the peak currents recorded in response to depolarization in high-potassium solution, as shown in Fig. 1. In two experiments, increasing the external concentration from 2 to 10 mM did not affect the magnitude of the inward current measured in high-potassium solution. Application of 100 μ M cadmium decreased the magnitude of both inward and outward currents by the same amount, \sim 10%, over the entire voltage range between -20 and $+50$ mV, even though the calcium current through any extant channels would be expected to vary greatly in this potential range. Thus, while these experiments cannot rule out the presence of some residual calcium current in the membrane currents reported here, they suggest that calcium currents must constitute <10% of the ionic current in our records, and may in fact make a considerably smaller contribution to the total current.

Calcium-activated potassium currents are also largely eliminated under these conditions. Our internal solutions contained high concentrations of fluoride and a calcium buffer (either EGTA or BAPTA) to maintain internal calcium concentrations at low levels to inhibit activation of calcium-activated potassium currents. While the presence of these calcium buffers is sufficient to maintain steady state internal calcium at low levels, there remains the possibility that, because of the finite time it takes for the buffering process, calcium levels near the membrane could transiently rise to high enough levels to activate the calcium-activated currents (Marty and Neher, 1985). However, we see no evidence of these currents in our records, which never showed the "N-shaped" dependence of potassium current on membrane voltage that is usually observed in cells having significant calcium-activated currents (Dubinsky and Oxford, 1984; Marty and Neher, 1985; Ritchie, 1987). In addition, we find that the potassium currents present under these conditions are relatively insensitive to block by TEA, with 95% of the current remaining unblocked in the presence of 5 mM TEA at -30 mV. In contrast, all of the potassium current at -30 mV was blocked by 5 mM 4-AP. Block of potassium currents was somewhat greater for TEA and less for 4-AP at more positive potentials. These data indicate that the bulk of our currents measured in high-potassium solution is carried by voltage-activated potassium channels.

RESULTS

Effects of Pandinus Venom

Fig. 2 shows current records from a GH₃ cell recorded in high-potassium solution with and without *Pandinus* venom. In the control records, potassium currents activate with depolarizations to potentials positive to -40 to -20 mV, reverse direction at ~ 0 mV, near E_K in these solutions, and inactivate with sustained depolarization. These properties are similar to those described previously for the voltage-gated potassium channels in GH₃ cells (Dubinsky and Oxford, 1984; Ritchie, 1987), with the differences that activation is apparent at more negative potentials in the high-potassium solutions we used and that inactivation of the potassium currents in some cells is complete with sustained depolarization under our ionic conditions. Replacing

the sodium in the normal external solution with potassium resulted in an increase in the potassium conductance, with this effect being greatest at negative potentials. The potassium conductance measured at -20 mV increased 5–15-fold in high-potassium solution relative to sodium Ringer. At $+50$ mV, there was an average 2.3-fold increase (± 0.5 , $n = 5$) in the peak potassium conductance in high potassium. These effects of external potassium on voltage-activated potassium currents are similar to those previously reported for potassium channels in myelinated axons (Dubois and Bergman, 1977). As has been reported previously in sodium external solutions, the amount of inactivating current in GH_3 cells varies from cell to cell. However, since we

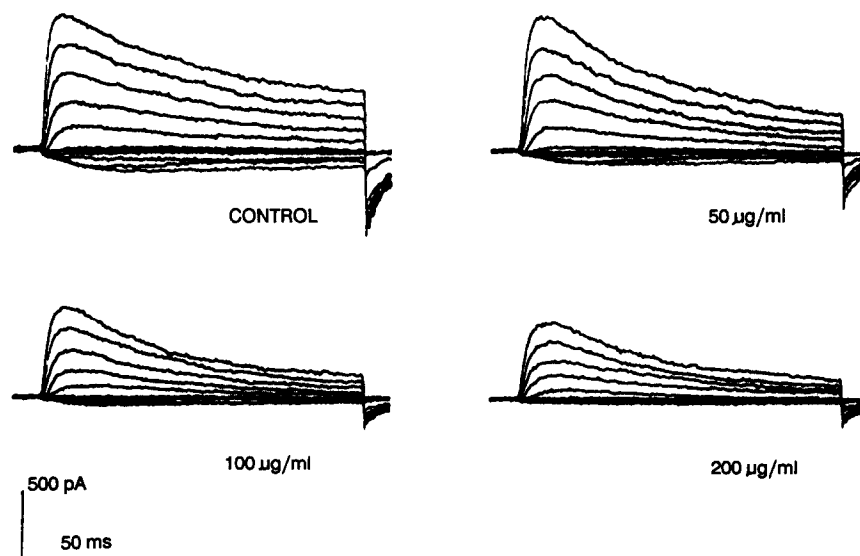


FIGURE 2. Effects of *Pandinus* venom on potassium currents in a GH_3 cell. Membrane currents were recorded in high-potassium solution before exposure to *Pandinus* venom and in the presence of 50, 100, and 200 $\mu\text{g}/\text{ml}$ *Pandinus* venom. Membrane currents were recorded during 200-ms depolarizations from a holding potential of -80 mV to potentials from -50 to $+50$ mV in increments of 10 mV. The control records were made 18 min after going to the whole-cell configuration. Venom solutions were applied in the order of increasing venom concentration. Currents in the venom solutions were recorded 7–10 min after each solution change. Cell PV31-1.

believe that calcium-activated currents are largely eliminated in our experiments (see Methods), we interpret these differences as being due to differences in voltage-gated potassium currents, rather than different proportions of voltage-activated and calcium-activated potassium currents.

Fig. 2 shows the effects of increasing concentrations of *Pandinus* venom on potassium currents in the same GH_3 cell. Two effects of the venom are apparent from these records. First, the potassium current is blocked by *Pandinus* venom in a dose-dependent fashion; second, block of potassium currents is more effective at negative membrane potentials than at positive potentials. Thus, under our ionic conditions, inward currents are blocked more completely than are outward currents. This can be

seen as a voltage-dependent effect of the venom rather than as an effect dependent on the direction of current flow through potassium channels, since the inward tail currents following repolarization of the membrane to the holding potential of -80 mV remain unblocked, even at a venom concentration that completely blocks the inward currents seen at depolarizations negative to 0 mV. In addition, the voltage dependence of venom block was also present in normal sodium-containing external solution, in which all potassium currents were outward.

Fig. 3 shows peak current-voltage relations and conductance-voltage relations derived from the data of Fig. 2. Increasing the concentration of venom decreases the amplitude of potassium currents, apparently by producing a decrease in the maxi-

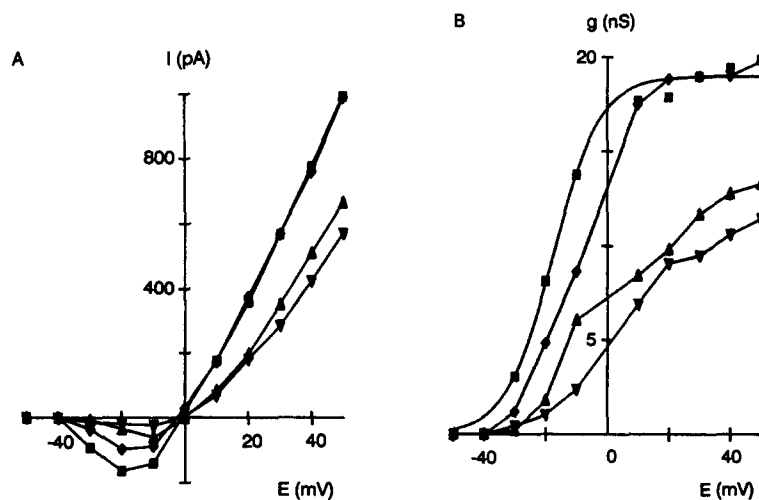


FIGURE 3. Effects of *Pandinus* venom on the voltage dependence of potassium current activation. (A) Peak current as a function of membrane potential in 0 (squares), 50 $\mu\text{g/ml}$ (diamonds), 100 $\mu\text{g/ml}$ (triangles), and 200 $\mu\text{g/ml}$ (inverted triangles) *Pandinus* venom. (B) Conductance-voltage relations from the peak currents in A, with the same symbols as in A. For the control data, the line is a Boltzmann function of the form $G = G_{\text{max}}/[1 + \exp\{(E_m - E_{1/2})/k\}]$, least-squares fitted to the control data with $G_{\text{max}} = 19.6$ nS, $E_{1/2} = -16.4$ mV, and $k = 8.0$ mV. Same cell as Fig. 2.

mum potassium conductance that can be elicited with strong depolarizations, by shifting the voltage dependence for activation of the potassium currents to more depolarized potentials, and by decreasing the voltage dependence of channel activation. The shift is apparent whether the voltage dependence of potassium current activation is calculated from the peak current during the depolarizing pulse, as was done in Fig. 3, or the tail current amplitude following the pulse is measured. The potential for half-activation of potassium currents measured from peak conductance during the pulse is shifted by $+16$ mV (± 2 [SEM, $n = 9$]) in 100 $\mu\text{g/ml}$ of venom relative to control values, while the slope parameter of the Boltzmann relations (k in the legend to Fig. 3) fitted to the same data increased by $40 \pm 14\%$.

The rates of activation and deactivation of potassium currents are also affected by

Pandinus venom. Fig. 4 A shows the time constants measured by fitting a single-exponential function to the potassium currents elicited during repolarization to the potentials shown after a depolarization to 0 mV. These tail currents were well-fitted by a single-exponential function, which is consistent with there being a single population of channels present in these experiments. *Pandinus* venom speeded the

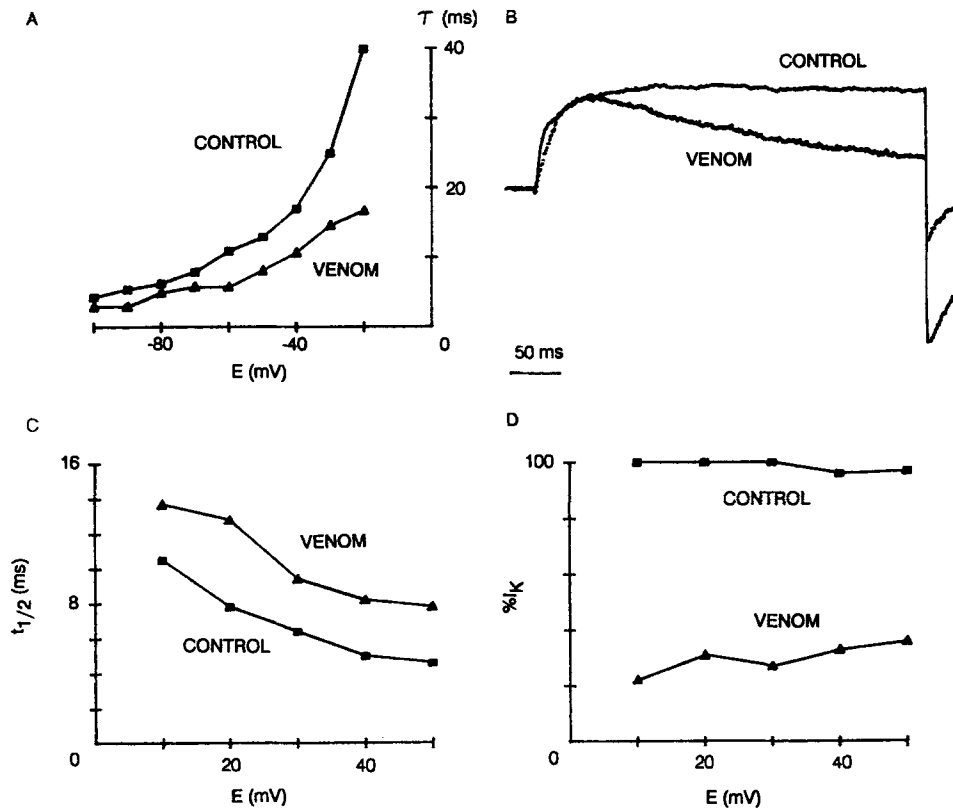


FIGURE 4. Effects of *Pandinus* venom on potassium current kinetics. (A) Tail time constants. Potassium channels were activated by a 15-ms depolarization to 0 mV. The decline of potassium current during repolarization to the potential shown was fitted by eye with a single-exponential function with the time constant (τ) shown. The squares are control records. The triangles are from currents recorded after 5 min in 100 $\mu\text{g/ml}$ *Pandinus* venom. Cell PV13-1. (B) Currents recorded at +50 mV before (solid line) and after (dotted line) exposure to 100 $\mu\text{g/ml}$ venom. The record in venom was scaled by a factor of 1.6 to have the same peak amplitude as the control. Cell PV25-1. (C) Time for half-activation. The symbols represent the time it took for the potassium current to reach half its peak value after depolarization to the potentials shown. The squares are control values. The triangles are from records taken after 8 min exposure to 100 $\mu\text{g/ml}$ *Pandinus* venom. Cell PV33-1. (D) Inactivation of potassium currents. The symbols represent the percent of the peak potassium current remaining at the end of a 200-ms depolarization to the potentials shown. A value of 100% indicates that there was no inactivation of the current. The squares are from measurements in the absence of venom. The triangles are from currents recorded after 2 min exposure to 100 $\mu\text{g/ml}$ *Pandinus* venom. Same cell as B.

deactivation of potassium currents at all potentials. The change in channel closing kinetics was equivalent to a shift in the potential dependence of channel closing of ~ 15 mV in this experiment. In other experiments, the effect of *Pandinus* venom was generally smaller than is seen in Fig. 4 A, although it was always in the direction to speed channel closing at any given membrane potential. *Pandinus* venom had a greater effect on channel opening rates. Opening was slowed by the venom, as illustrated in Fig. 4 C, which shows the time it took the potassium current to reach half its maximum value after depolarization in the control and in 100 $\mu\text{g/ml}$ venom. The slowing of potassium channel activation produced by the venom was equivalent to a shift in the voltage dependence of channel activation kinetics of ~ 30 mV to more depolarized potentials in this experiment. This experiment showed the largest effects seen on the activation rates. The average apparent shift in the voltage dependence of channel activation rates in 100 $\mu\text{g/ml}$ venom was 19 mV (± 2 mV, $n = 9$) in these experiments.

Pandinus venom also affected the extent to which potassium currents inactivated during depolarizing voltage pulses. Fig. 4 B shows current records during a depolarization to +50 mV under control conditions and after exposure to 100 $\mu\text{g/ml}$ venom, scaled to have the same peak amplitude. In the control record, there was no inactivation of the potassium current in this cell during the 200-ms depolarization. The amount of inactivation of potassium currents seen in our GH₃ cells was highly variable, ranging from 0 to 71% of the peak current inactivated at 200 ms. *Pandinus* venom increased the amount of inactivation of potassium currents, as illustrated in Fig. 4, B and D. The amount of peak potassium current inactivated by a 200-ms depolarization increased from an average of 36% ($\pm 8\%$, $n = 9$) in the controls to 64% ($\pm 11\%$, $n = 9$) in the presence of 100 $\mu\text{g/ml}$ venom. The amount of inactivation induced by the venom was highly variable, ranging from 0% (in a cell in which 40% of the control current inactivated) to 61% (in a cell that initially showed no inactivation). There was no apparent voltage dependence of the amount of inactivation measured this way and the proportion of the peak potassium current that inactivated during depolarizations was independent of membrane potential both in the controls and in the presence of venom, as shown in Fig. 4 D.

Time Course of Venom Effects

Block of potassium currents by *Pandinus* venom occurs rapidly after exposure of the cell to the venom, and was not reversible on the time scale of our experiments. Fig. 5 shows the peak potassium current measured at +30 mV before, during, and after exposure to 100 $\mu\text{g/ml}$ *Pandinus* venom. The onset of venom block of the potassium current occurs rapidly, with the initial time course possibly dependent on the rate of exchange of solution in the experimental chamber. A second component of block is apparent in the decline of current that occurs over the next several minutes. The block of potassium currents reached an apparent steady state within ~ 10 min. Washout of the venom failed to reverse the block of potassium currents by *Pandinus* venom, even at long times after removal of the venom. In six experiments examining the reversibility of venom effects, there was little or no recovery from the venom block 15–40 min after washout of the venom, following exposure of the cells to venom concentrations between 50 and 500 $\mu\text{g/ml}$. Often there was a slight, transitory

increase in current amplitudes in the minutes immediately after the solution change, which could amount to as much as 15% recovery of current amplitude. However, as seen in Fig. 5, this slight recovery was not sustained. Control experiments examining the stability of potassium currents under our experimental conditions showed little variation in current amplitudes or voltage dependence with time, with the peak potassium current remaining within 10% of its initial value after >1 h under voltage clamp. We conclude from these results that *Pandinus* venom rapidly blocks the potassium currents of GH₃ cells, and that block by the venom is slow to reverse. However, because the crude venom used in these experiments contains at least two

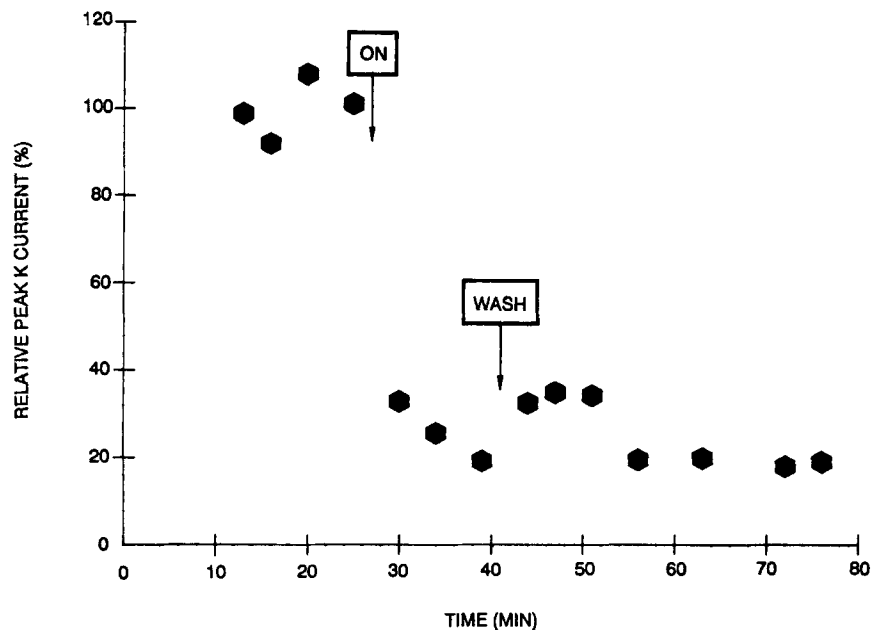


FIGURE 5. Time course of venom effects. The block of potassium currents by *Pandinus* venom is rapid and irreversible. Peak current amplitude, expressed as the percent of the average of the control values, was measured during a depolarization to +30 mV before, during, and after exposure to 100 $\mu\text{g}/\text{ml}$ venom. The venom solution was washed on at the "on" arrow and washed off at the "wash" arrow. The cell was in the venom solution for 15 min before the venom was washed out. Cell PV34-2.

components that affect potassium currents (see *Effects of Zinc* below), we cannot ascribe the time course of venom effects we observed to the binding of a single molecular entity.

Concentration Dependence of Pandinus Venom Effects

Fig. 6 shows the concentration dependence of potassium current block by *Pandinus* venom measured at two different voltages, -20 and $+50$ mV. Current amplitude measurements were made in venom solutions after 9–15 min in the solution to allow the level of block to reach an apparent steady state value. Shorter exposures to venom

resulted in less apparent block of the current. Potassium currents are blocked more completely at the more negative membrane potentials, as discussed above. However, at neither potential is block of potassium currents complete, even at a 10-fold-higher concentration of venom than the 50 $\mu\text{g}/\text{ml}$ that blocks 50% of the current at -20 mV. Since 20% of the potassium current at -20 mV is resistant to block by *Pandinus* venom, this would indicate that the crude venom blocks the sensitive currents, with a dissociation constant of <50 $\mu\text{g}/\text{ml}$.

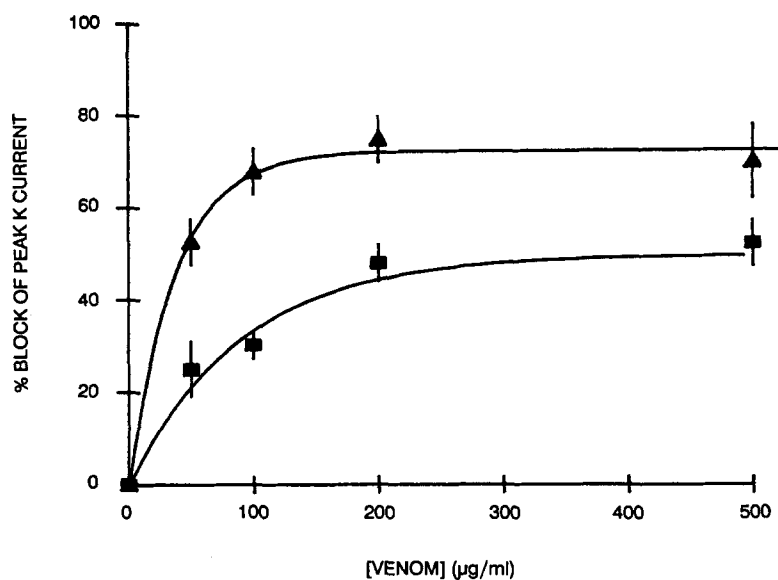


FIGURE 6. Concentration dependence of the block of potassium currents by *Pandinus* venom measured at -20 mV (triangles) and $+50$ mV (squares). Block is more complete at more negative membrane potentials. The percent block of potassium current derived from the peak current measured in venom relative to control values measured before venom application is plotted. Control measurements were made immediately before venom application and 18–44 min after going to the whole-cell configuration. Measurements in venom solutions were made 9–15 min after the switch to venom-containing solution. Each point represents the average of three to nine measurements in different cells. The error bars represent the standard error of the mean. Some cells were exposed to more than one concentration of venom, always in the order of increasing venom concentration.

Effects of Zinc

Pandinus venom contains appreciable quantities of zinc: $\sim 2 \times 10^{-7}$ mol/mg (Pappone and Cahalan, 1987). We tested the effects of zinc on the potassium currents of GH_3 cells in order to determine whether the effects of *Pandinus* venom could be due to the zinc it contains. We found that the presence of zinc has effects on potassium currents in GH_3 cells that are very similar to those of *Pandinus* venom, but differ from the venom in that they are readily reversed. Fig. 7 A shows the effects of zinc on the potassium currents. Zinc, at a concentration of 0.2 mM, blocks a substantial portion

of the potassium current, with the greatest degree of block occurring at negative membrane potentials. Like *Pandinus* venom, zinc caused a slowing of the turn-on of potassium currents with depolarization and a speeding of the turn-off of currents with repolarization. In addition, zinc shifted the voltage dependence of peak potassium current activation to more positive potentials by 6–14 mV. These effects of zinc on the potassium currents of GH₃ cells are similar to those observed on neuronal and muscle potassium currents (Stanfield, 1975; Århem, 1980; Gilly and Armstrong, 1982; Pappone and Cahalan, 1987). All these effects of zinc are similar to the effects of *Pandinus* venom on potassium currents. However, in contrast to the irreversible

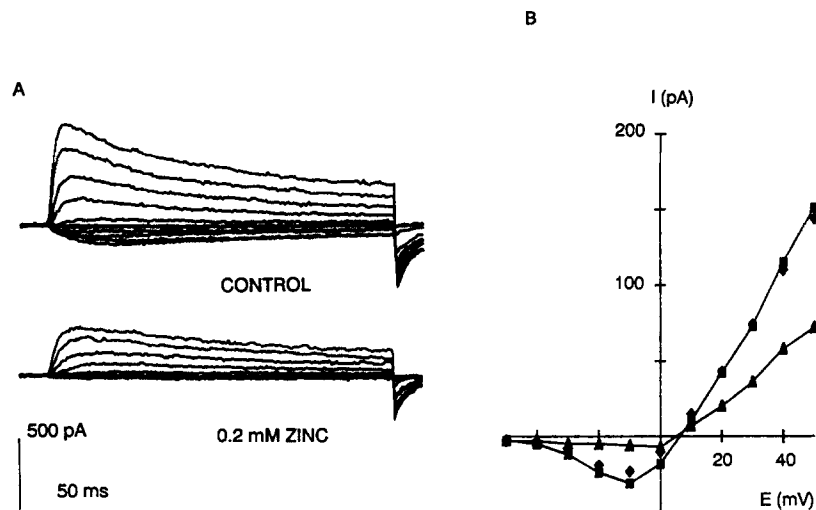


FIGURE 7. Zinc reversibly blocks potassium currents. (A) Membrane currents recorded in high-potassium solution before and during exposure to 0.2 mM zinc. Membrane currents were recorded during 200-ms depolarizations from a holding potential of -80 mV to potentials from -50 to $+50$ mV in increments of 10 mV. The control records were made 29 min after going to the whole-cell configuration. Cell PV12-2. (B) Peak current-voltage relations measured in high-potassium solution before (squares), during (triangles), and after (diamonds) exposure of the cell to 0.2 mM zinc. The control records were made 29 min after going to the whole-cell configuration. The cell was in the zinc solution for 16 min. The records after zinc exposure were made 8 min after washout of the zinc. Same cell as in A.

nature of the venom effects, the effects of zinc were quickly and completely reversed by washout. Fig. 7 B shows peak potassium current amplitudes measured in a GH₃ cell before, during, and after exposure to 0.2 mM zinc. This concentration is twice that we would expect to be present in the highest concentration of venom used in these experiments, 500 μ g/ml. Potassium currents are blocked in the presence of zinc, but even after an 11-min exposure to the zinc, the potassium current recovered to 90% of its control values within 2 min, and recovered completely within 8 min after washing out the zinc. Increasing the zinc concentration to 2 mM increased the blocking effects further and slowed their reversal. However, in contrast to our results with

Pandinus venom, >60% of the control potassium current returned within 2–3 min of washing out even these high concentrations of zinc.

Effects of Pandinus Venom on Sodium and Calcium Currents

Membrane currents were measured in a zero-potassium sodium external solution with cesium as the major internal cation in the pipette in order to measure venom effects on sodium and calcium currents in the absence of potassium currents. In three experiments, crude venom in concentrations of 100–200 $\mu\text{g/ml}$ blocked 12–24% of the peak inward sodium current. Block of sodium currents by venom was accompanied by a shift of the potential dependence of sodium current activation by 6–7 mV toward more depolarized potentials. The potential dependence of steady state inactivation of the sodium current was unaffected by the venom. The effects of *Pandinus* venom were not rapidly reversible, although recovery was only monitored for a few minutes after washout of the venom. These results differ from those in myelinated nerve fibers, where *Pandinus* venom at similar concentrations had no effect on sodium currents (Pappone and Cahalan, 1987). The effects of crude venom on sodium currents seem to be due to venom components other than those affecting potassium currents, since a partially purified fraction of the venom that blocks potassium currents had no effect on the sodium current magnitude or the voltage dependence of sodium current activation. One possibility is that the sodium current effects are due to the zinc present in the crude venom, which is known to decrease sodium conductance and shift its activation in other cell types (Århem, 1980; Gilly and Armstrong, 1982).

Pandinus venom also decreased calcium currents in these cells, although it was difficult to quantify this effect reliably in the face of the rapid rundown of calcium currents under our experimental conditions. Comparing currents immediately before and after exposure to 100 $\mu\text{g/ml}$ venom suggests that ~20% of the peak calcium current could be rapidly blocked by *Pandinus* venom. This effect was independent of membrane potential.

DISCUSSION

This article describes the effects of *Pandinus imperator* scorpion venom on the voltage-gated potassium currents of voltage-clamped cells from a rat anterior pituitary tumor cell line, GH₃ cells. We find that *Pandinus* venom blocks the potassium channels of GH₃ cells with a concentration dependence for block of half the potassium current in the range of 10–50 $\mu\text{g/ml}$. Block of potassium currents by *Pandinus* venom was largely irreversible and was highly dependent on membrane potential, with potassium currents much more completely blocked at negative membrane potentials than at positive potentials. These features of *Pandinus* venom's actions on GH₃ cells are essentially the same as those observed in frog myelinated nerve fibers (Pappone and Cahalan, 1987).

Several other scorpion venoms (Koppenhoefer and Schmidt, 1968; Narahashi et al., 1972; Carbone et al., 1983) and scorpion venom-derived toxins (Romey et al., 1975; Possani et al., 1981, 1982; Carbone et al., 1982, 1983) have been described that act on the voltage-activated potassium currents of neurons. Block of potassium

currents by these agents differs from that produced by *Pandinus* venom in that the block is independent of membrane voltage and is readily reversed upon washout of the agent. The actions of *Pandinus* venom more closely resemble the effects of a number of sodium channel scorpion toxins, which act irreversibly to shift the voltage dependence of sodium channel gating. The effects of *Pandinus* venom in myelinated nerve are very similar to those of a toxin derived from black mamba venom, dendrotoxin (Benoit and Dubois, 1986). Dendrotoxin appears to act on the same subpopulation of voltage-activated potassium channels as *Pandinus* venom does in myelinated nerve. This subpopulation of potassium channels, termed f_1 (Dubois, 1981), activates at relatively negative membrane potentials and has rapid kinetics. In hippocampal neurons, dendrotoxin blocks A currents (Dolly et al., 1984), a class of inactivating potassium channels involved in determining the rates of repetitive firing in neurons (Conner and Stevens, 1971).

Our results would also be consistent with *Pandinus* venom acting on a subpopulation of potassium channels in GH_3 cells that was activated at more negative membrane potentials and had different kinetics than the residual unblocked channels. Multiple types of potassium channels with differing pharmacologies are known to be present in GH_3 cells (Dubinsky and Oxford, 1984; Ritchie, 1987). If *Pandinus* venom is blocking a subset of potassium channels in GH_3 cells, then the blocked and unblocked potassium channels would represent previously uncharacterized subcategories of voltage-gated potassium channels, differing in their voltage dependence for activation and in their kinetics. We have no kinetic evidence for multiple types of voltage-activated potassium currents in our experiments, since conductance-voltage relations were always monotonic and the decay of tail currents could be well fitted by a single-exponential function. However, the fact that potassium currents were only partially susceptible to block by all three pharmacological agents used in these experiments—TEA, 4-AP, and *Pandinus* venom—may be evidence of the presence of multiple types of voltage-dependent potassium channels in GH_3 cells. These results could indicate that the voltage-gated potassium channels blocked by dendrotoxin and *Pandinus* venom (A channels of nerve cell bodies, the f_1 fraction of axonal potassium channels, and the voltage-gated potassium channels of GH_3 cells) may be closely related molecular entities, although differences in their pharmacology and kinetics indicate that they are not identical.

Alternatively, it is possible that *Pandinus* venom does not block a subpopulation of potassium channels, but rather acts to alter the gating properties of the channels. In this scenario, *Pandinus* venom shifts the potential dependence of channel kinetics to more depolarized potentials. In this context, the similarities between the effects of *Pandinus* venom and zinc on the potassium currents are intriguing. The effects of zinc on GH_3 cells are similar to those seen in squid axon (Gilly and Armstrong, 1982). It has been proposed, based on the effects of zinc and other divalents on potassium currents, that external divalent cations play a pivotal role in potassium channel gating (Gilly and Armstrong, 1982; C. M. Armstrong and Matteson, 1986; C. M. Armstrong and Lopez-Barneo, 1987). Divalent cations, normally calcium, are proposed to stabilize the closed conformation of voltage-gated potassium channels and to contribute to the voltage dependence of the channel kinetics. Zinc is envisioned as acting more effectively than calcium in this role, thus promoting channel closure and inhibiting

channel opening. *Pandinus* venom contains appreciable quantities of zinc, and the effects of venom in GH₃ cells are in all respects similar to those of zinc except that they are irreversible. It is possible, therefore, that *Pandinus* venom acts to stabilize the binding of zinc, and possibly other divalent cations as well, to regulatory sites on the potassium channel, and thus shifts the gating parameters of potassium channels. If so, the active components of the venom would have to interact strongly with the channel to account for the differences in the time courses of recovery from zinc and *Pandinus* venom exposure. This interaction then could prevent the normal fast unbinding of zinc from the site, or possibly promote a similar strong binding of calcium or other divalent cations to the site.

Both the irreversibility of *Pandinus* venom effects and the concentration dependence of its action suggest that the toxins contained in the venom bind with high affinity to potassium channels. The dose-response relation measured for *Pandinus* venom (Fig. 6) indicates that 16 µg/ml of crude venom is sufficient to block half the potassium current susceptible to block at a membrane potential of -20 mV. A dissociation constant for the toxins in *Pandinus* venom in the nanomolar range can be calculated from these data, assuming that 95% of the crude venom consists of inactive mucoid proteins (Chen and Pappone, unpublished observations) and that the toxin has a molecular weight in the range of 4,000–7,000 daltons, similar to other scorpion-derived potassium channel neurotoxins (Possani et al., 1981, 1982; Miller et al., 1985). Thus, the affinity is such that the toxin could be a valuable tool for studying the properties and distribution of voltage-gated potassium channels.

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