Manipulation of the Potassium Channel Kv1.1 and Its Effect on Neuronal Excitability in Rat Sensory Neurons

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Chi XX, Nicol GD. Manipulation of the potassium channel Kv1.1 and its effect on neuronal excitability in rat sensory neurons. J Neurophysiol 98: 2683–2692, 2007. First published September 12, 2007; doi:10.1152/jn.00437.2007. Potassium channels play a critical role in regulating many aspects of action potential (AP) firing. To establish the contribution of the voltage-dependent potassium channel Kv1.1 in regulating excitability, we used the selective blocker dendrotoxin-K (DTX-K) and small interfering RNA (siRNA) targeted to Kv1.1 to determine their effects on AP firing in small-diameter capsacin-sensitive sensory neurons. A 5-min exposure to 10 nM DTX-K suppressed the total potassium current ($I_K$) measured at $+40$ mV by about 33%. DTX-K produced a twofold increase in the number of APs evoked by a ramp of depolarizing current. Associated with increased firing was a decrease in firing threshold and rheobase. DTX-K did not alter the resting membrane potential or the AP duration. A 48-h treatment with siRNA targeted to Kv1.1 reduced the expression of this channel protein by about 60% as measured in Western blots. After treatment with siRNA, $I_K$ was no longer sensitive to DTX-K, indicating a loss of functional protein. Similarly, after siRNA treatment exposure to DTX-K had no effect on the number of evoked APs, firing threshold, or rheobase. However, after siRNA treatment, the firing threshold had values similar to those obtained after acute exposure to DTX-K, suggesting that the loss of Kv1.1 plays a critical role in setting this parameter of excitability. These results demonstrate that Kv1.1 plays an important role in limiting AP firing and that siRNA may be a useful approach to establish the role of specific ion channels in the absence of selective antagonists.

INTRODUCTION

Voltage-gated potassium channels (Kv) are protein complexes consisting of ion-conducting integral protein α-subunits and auxiliary cytoplasmic β-subunits. These Kv channels are quite diverse due to numerous gene families, heteromultimeric combination of α-subunits, auxiliary subunits, splice variants, and post-translational processing. More than a dozen α-subunits of the Kv superfamily have been isolated and have been divided into several subfamilies, Kv1–6, 8, and 9 (Chandy and Gutman 1995; Gutman et al. 2003, 2005; Pongs 1992). Functionally, Kv channels play an important role in setting the resting membrane potential, in controlling repolarization of the action potential (AP), and in modulating the frequency of firing. However, the contribution that a specific channel subtype of the Kv family makes to a particular physiological aspect of neuronal membrane properties or AP firing is poorly understood because of the coexpression of multiple subtypes in any given neuron, the capacity of the α-subunits to form heteromultimers, and the paucity of specific blockers and antagonists.

The α-subunits of the Kv family are mammalian homologues of the Shaker subunits originally described in Drosophila (see Baumann et al. 1988; Schwarz et al. 1988; Tempel et al. 1987, 1988). Multiple members of the Kv subfamily (1.1–1.6) have been found in the CNS (Coetzee et al. 1999). The α-subunits of Kv1 are known to form heteromultimers (Christie et al. 1990; Isacoff et al. 1990; Ruppersberg et al. 1990; Wang et al. 1993). Studies have demonstrated that in the nervous system Kv1.1 can combine with Kv1.2, Kv1.3, and Kv1.6, or Kv1.4 (Scott et al. 1994; Wang et al. 1999). However, homomers of Kv1.1 have not been detected in the nervous system (Scott et al. 1994; Shamotienko et al. 1997; Wang et al. 1999). In contrast, when expressed in heterologous cell systems, Kv1.1 gave rise to a rapidly activating, sustained outward current (Bosma et al. 1993; Robertson and Owen 1993; Stuhmier et al. 1988). In the nervous system, Kv1.1 appears to be widely distributed; it is highly expressed in the juxtaparanodal regions of myelinated axons. However, the channel is also expressed in unmyelinated axons, cell somas, axon terminals, and in some dendrites (Wang et al. 1993, 1994). Disruption of the Kcnal gene (the gene locus for Kv1.1) has significant behavioral consequences. These Kv1.1 knock-out mice displayed frequent spontaneous seizures, which begin to appear about 3 wk postnatally with about half of the mice dying suddenly between week 3 and week 5. Those mice that lived to adulthood continued to display spontaneous seizures (Rho et al. 1999; Smart et al. 1998). Recordings from neurons in Kcnal-null animals demonstrated that less current was required to produce AP firing and that these Kv1.1-null neurons typically fired many APs compared with the single AP observed in wildtype mice (Brew et al. 2003; Smart et al. 1998). These results indicate that Kv1.1 plays a critical role in setting both the point at which a neuron generates an AP and for a given stimulus whether the cell fires single or multiple APs.

Early studies in sensory neurons isolated from either dorsal root ganglia (DRG) or nodose ganglia showed that dendrotoxin inhibited a slowly inactivating outward $I_K$ (Penner et al. 1986; Stansfeld et al. 1986, 1987, 1988). Current-clamp recordings demonstrated that in rat sensory neurons isolated from the nodose ganglia, exposure to α-dendrotoxin (α-DTX), an antagonist for the α-subunits of Kv1.1, Kv1.2, and Kv1.6 (Grissmer et al. 1994; Grue et al. 1990; Harvey and Karlsson 1980), lowered the firing threshold of the AP and increased the frequency of firing (Glazebrook et al. 2002; Stansfeld et al. 1986). Similarly, in neurons isolated from trigeminal ganglia, α-DTX increased the number of APs evoked by a step of
current without changing the resting membrane potential (Yoshida and Matsumoto 2005). In addition, the Kv1 family appears to play an important role in modulating neuronal activity under pathological conditions. In rat sensory neurons of the lumbar DRG, hypoxia increased the excitability through a suppression of α-DTX-sensitive potassium currents (Gruss et al. 2006). Taken together, these findings suggest that the Kv1.1, Kv1.2, and Kv1.6 subtypes likely play an important role in regulating cell excitability due to the suppression of α-DTX-sensitive potassium currents under normal as well as pathological conditions. However, due to the expression of multiple channel subtypes as well as their capacity to form heteromultimers, it is not clear which specific subtypes function in modulating particular aspects of excitability in sensory neurons.

To address this question, we used the specific blocker of Kv1.1, dendrotoxin-K (DTX-K, IC50 ~2 nM; Owen et al. 1997; Robertson et al. 1996) and small interfering RNA (siRNA) targeted to Kv1.1 to specifically reduce the expression of this channel and thereby determine the role of Kv1.1 in modulating the excitability of small-diameter capsaicin-sensitive sensory neurons. In this report, we demonstrate that exposure to DTX-K augmented the number of APs evoked by a ramp of current and that this increased excitability was associated with changes in specific parameters of the AP, whereas other properties were unaffected. Reduction of Kv1.1 protein with siRNA produced changes that were consistent with the acute effects of DTX-K. These results suggest that Kv1.1 plays a significant role in regulating the firing properties of sensory neurons.

METHODS

Isolation and maintenance of adult rat sensory neurons

Sensory neurons were isolated from young adult rats using procedures described by Lindsay (1998) with slight modifications. Briefly, male Sprague–Dawley rats (100–150 g) were killed by placing them in a chamber filled with CO2. The DRG were collected in a chamber where neurons were bathed in normal Ringer solution of the following composition (in mM): 140 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 5 EGTA (calculated free Ca2+ concentration of ~100 nM; MaxChelator), and 10 HEPES (pH was adjusted to 7.3 with KOH). This pipette solution was also used in the current-clamp recordings. The membrane was held at ~60 mV; this value was chosen so that current measurements could be ascertained at a voltage that reflected the normal resting potential in these sensory neurons. Activation of IK was determined by 100-ms voltage steps, applied at 3-s intervals in +10-mV increments from +80 to +40 mV. At the end of these steps, the voltage was set to +40 mV for 20 ms, after which it returned to the holding potential of ~60 mV. The currents were filtered at 5 kHz and sampled at 1 kHz using pClamp 8.0 (Molecular Devices). After obtaining the control response, the bath solution was changed to the appropriate Ringer solution and cells were superfused continuously for the appropriate times. In a separate series of time control experiments, the maximum current amplitudes during the voltage step for IK did not vary significantly over a 20-min time period, indicating that there was little rundown of this current over this time. At the end of each recording, the neuron was exposed to 100 nM capsaicin. This neurotoxin was used to distinguish capsaicin-sensitive sensory neurons because these neurons are believed to transmit nociceptive information (Holzer 1991). However, the correlation between capsaicin sensitivity and that a neuron is a nociceptor is not absolute. Some nociceptive neurons are insensitive to capsaicin and some capsaicin-sensitive neurons are not nociceptors (see Petruska et al. 2000). Therefore this agent was used to define a population of small-diameter sensory neurons that could serve a nociceptive function. The results subsequently reported were obtained from capsaicin-sensitive neurons only. All experiments were performed at room temperature (~22°C).

Data analysis

Data are presented as means ± SE. The AP firing threshold was determined by differentiating the voltage trace (dv/dt) evoked by the ramp in current-clamp recordings. The voltage and time at which the first AP was fired were taken as the point that exceeded the baseline value of dv/dt by >20-fold. The baseline value of dv/dt was determined by averaging the points over 100 ms that began with the onset of the current ramp (65–165 ms). The rheobase was measured as the amount of ramp current at the firing threshold. To evoke a single AP (sampled at 10 kHz), steps of current in 40-pA increments and 25 ms in duration were used. The duration of the AP was measured at half the total amplitude of the AP. Statistical differences between the control recordings and those obtained under various treatment conditions were determined by using either a t-test, paired t-test, ANOVA, or repeated-measures (RM) ANOVA. When a significant difference was obtained with an ANOVA, post hoc analyses were performed using a Tukey test. Values of P < 0.05 were judged to be statistically significant.
The presence of gene transcripts for Kv1.1 was detected after electrophysiological recording using techniques described by Song et al. (1998) with modification. Briefly, $I_K$ was recorded from a small-diameter sensory neuron; the cell was aspirated into another sterilized micropipette containing 5 μl DEPC (diethylpyrocarbonate)-treated water. The contents of the micropipette were forced into a microtube and the RNA was reverse transcribed using the SuperScript first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacturer’s instructions. The cDNA was stored at −20°C before the PCR detection and amplification of Kv1.1 using the forward primer (bases 1573–1595): CCG CCG CAG CTC TTC TAT CA and the reverse primer (bases 1781–1758): CAA GGG TTT TGT TTG GGG GCT TTT using the Platinum PCR Supermix (Invitrogen). These PCR reactions ran for 45 cycles (94°C for 1 min, 51°C for 1 min, 72°C for 2 min). The PCR product was sequenced using an ABI Prism 3100 genetic analyzer at facilities in the Department of Biochemistry and Molecular Biology, Indiana University School of Medicine.

Immunohistochemistry

Fluorescence labeling of Kv1.1 was performed in isolated sensory neurons. Isolated neurons were grown on glass-bottom microwell dishes (MatTek, Ashland, MA) for 48 h and were washed with PBS without Ca$^{2+}$ or Mg$^{2+}$ (PBS-CM, 0.1 M, pH 7.4). The cells were fixed with 4% paraformaldehyde for 10–15 min and permeabilized with 0.025% saponin for 2 min at room temperature. After extensive washing with PBS-CM, cells were blocked with 2% bovine serum albumin and 0.025% saponin and fish skin gelatin in PBS-glycine (10 mM) for 5 min at room temperature. To detect Kv1.1, the cells were incubated with the primary antibody for Kv1.1 (1:100) overnight at 4°C. After washing in PBS-CM (three times, 5 min each), cells were incubated in darkness for 1 h at room temperature with a secondary anti-rabbit Cy5-conjugated antibody (1:200, Jackson Immuno Research, West Grove, PA) and washed in PBS-CM (three times, 5 min each). Fixed cells were immersed in SlowFade antifade reagent (Molecular Probes, Eugene, OR). Immunofluorescence was observed using confocal microscopy. Images were collected using a Zeiss LSM510 microscope equipped with a Zeiss ×63 water-immersion lens. Optimal images were obtained by averaging two scans. The excitation and emission wavelengths for Cy5 were 650 and 680 nm, respectively.

siRNA

An siRNA sequence corresponding to nucleotide positions 599–619 (AAA TTT TAC GAG TTG GGC GAG) of rat Kv1.1 mRNA (NM_173095) was selected according to the software provided by Invitrogen. The cells were rinsed once with Optimem media and incubated at 37°C for about 30 min. The Neuroporter–siRNA complex (100 nM) was added on day 3 in culture wherein the neurons were exposed to the siRNA or Neuroporter alone for 48 h at 37°C. After 2 days (day 5 in culture), the Neuroporter ± siRNA was washed out and the normal media containing antibiotics and NGF was then added to the neurons and allowed to incubate for another 2 days before electrophysiological recordings or Western blots were performed.

Western blot

Isolated sensory neurons either in the absence or presence of siRNA for Kv1.1 were sonicated in fresh TNN-SDS buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Nonidet P-40, 50 mM NaF, 20 mM EDTA, 1 mM sodium orthovanadate, 1 mM diithiothreitol, 0.1% SDS, and 2 mM phenylmethysulfonyl fluoride). The cell lysates were prepared in TNN-SDS buffer at 4°C for 30 min followed by centrifugation (10,000 g for 10 min). Protein concentration was measured using the Bradford method. Equivalent amounts of protein (30 μg) were loaded and separated on a NuPAGE 4–12% Bis-Tris Gel (Invitrogen) before transfer to a PVDF (polyvinylidene fluoride) membrane (Invitrogen) for Western blot analysis. After serial incubation with specific antibodies, immunoreactive bands on the membrane were developed by ECL kit (Amersham Biosciences, Piscataway, NJ) and visualized by exposure to Kodak LS X-OMAT film. The density of the bands was measured by Quantity One software (Bio-Rad Life Science Research, Hercules, CA).

Chemicals

Dendrotoxin-K and the primary antibody to Kv1.1 used in the immunohistochemical studies were obtained from Alomone Labs (Jerusalem, Israel). The primary antibody to Kv1.1 used in the Western blots was obtained from Sigma Chemical (St. Louis, MO). The primary antibody to Kv1.2 used in the Western blots was obtained from NeuroMab (Davis, CA). Tissue culture supplies were purchased from Invitrogen. All other chemicals were obtained from Sigma Chemical. Capsaicin was dissolved in 1-methyl-2-pyrrolidinone to obtain concentrated stock solutions. The stock solutions were then diluted with Ringer solution to yield the appropriate concentration.

We previously demonstrated that the vehicle, 1-methyl-2-pyrrolidinone, has no effect on AP firing or the activation of $I_K$ (Zhang et al. 2002).

RESULTS

Small-diameter sensory neurons express Kv1.1

To determine whether a small-diameter sensory neuron expressed the mRNA for Kv1.1, single-cell RT-PCR was used in combination with whole cell patch-clamp recording. The outward current obtained from a representative neuron is shown in Fig. 1 (top). These currents exhibited the rapid activation and little time-dependent inactivation that is characteristic of delayed rectifier potassium currents ($I_K$). These currents are similar to those observed when the cloned Kv1.1 was expressed in a heterologous expression system (see Fig. 1; Bosma et al. 1993). After recording the outward current, the neuron was aspirated into a pipette from which the single-cell RT-PCR was performed. As shown in the bottom panel of Fig. 1, lane A demonstrates that the PCR product obtained from the cDNA from five small-diameter sensory neurons was of the correct product size (208 bp). In addition, the PCR product was sequenced and found to exactly match the targeted bases. The PCR product for Kv1.1 obtained from the single neuron exhibiting the delayed rectifier-like current in the top panel (lane B) was at the same base-pair size, although less intense, as that obtained from the five neurons (lane A). Lane C indicates that in the absence of cDNA, no PCR product was obtained. In cDNA obtained from rat brain, a PCR product of 208 bp was also detected (data not shown). These results indicate that small-diameter sensory neurons express the mRNA for Kv1.1. To further support the RT-PCR measurements, immunohistochemistry was used to determine whether the Kv1.1 protein
was expressed in the neurons. Incubation with the Kv1.1 antibody stained small-, medium-, and large-diameter neurons with varying degrees of fluorescence intensity, although not every neuron expressed Kv1.1 (see Fig. 2, A–E). In the absence of primary antibody, no reaction was observed with the secondary antibody alone (see Fig. 2F). Also, expression of Kv1.1 was detected in Western blots obtained for the control conditions in the siRNA studies (see Fig. 4, top right). Taken together, these results demonstrate that sensory neurons express the potassium channel Kv1.1 and are consistent with previous reports (see DISCUSSION).

DTX-K increases the excitability of small-diameter capsaicin-sensitive sensory neurons

We sought to establish whether the specific blocker of Kv1.1, DTX-K, could alter the excitability of small-diameter capsaicin-sensitive sensory neurons. The current-clamp configuration was used to examine the effects of DTX-K on the capacity of sensory neurons to fire APs when stimulated with a ramp of depolarizing current. As shown for a representative neuron (Fig. 3A), a 5-min exposure to 10 nM DTX-K increased the number of APs evoked by the current ramp from a control value of 2 to 10 APs. The results for the current-clamp experiments obtained from a total of 11 small-diameter sensory neurons are summarized in Fig. 3B. Exposure to DTX-K significantly increased the number of APs from a control value of $2.5 \pm 0.3$ to $7.7 \pm 1.5$ APs (paired t-test) after a 5-min application. The increase in AP firing produced by DTX-K was accompanied by a significant hyperpolarizing shift of about 4 mV in the firing threshold and a 50% reduction in the rheobase (see Table 1, $n = 11$, paired t-test). However, neither the resting membrane potential nor the duration of the AP was altered by DTX-K (see Table 1). Most of these untreated neurons (7 of 11) exhibited an inflection (a hump) on the falling phase of the AP (assessed by $dV/dt$). Rat sensory neurons exhibiting this inflection were associated with slower conduction velocities (C and Aβ fibers; Harper and Lawson 1985) and are presumed to be characteristic of identified nociceptive neurons in both cat and rat (Koerber et al. 1988; Ritter and Mendell 1992). Thus these DTX-K–induced changes in the number of APs and the firing threshold are indicative of the important role that Kv1.1 plays in regulating each respective parameter and its contribution to the total excitability of these neurons.

siRNA treatment reduces the expression of Kv1.1

To establish the functional contribution of Kv1.1 to neuronal excitability, expression of this channel protein was reduced using siRNA targeted to the α-subunit of Kv1.1 (amino acids

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**FIG. 2.** Sensory neurons stain positive for Kv1.1. A–E: show that the protein for Kv1.1 was expressed in small-, medium-, and large-diameter sensory neurons. Top: fluorescence image. Bottom: bright-field image. These images were obtained from the same harvest of sensory neurons and were obtained at the same confocal settings. A: shows that a medium-diameter neuron is positive for Kv1.1, whereas the 2 smaller neurons are negative (indicated by the arrows). B: small-diameter neuron that is negative for Kv1.1. C and E: 2 small-diameter neurons wherein one is positive and one is negative (arrows) for Kv1.1. D: large-diameter neuron positive for Kv1.1 and a medium-diameter neuron that is negative (arrow). F: representative image obtained in the absence of primary antibody, indicating the specificity of this reaction. Scale bar in A represents 30 μm and applies to all panels.
599–619, from the origin). These results are summarized in Fig. 4. The top panels show representative Western blots obtained for dishes of isolated sensory neurons (~5,000 cells) undergoing three different experimental treatments. The right lane represents the untreated control condition wherein Kv1.1 was detected at the expected size of about 80 kDa in isolated sensory neurons that were in culture for 7 days, the same length of time as for the siRNA treatment. The middle lane represents the detection of Kv1.1 in neurons that were exposed to siRNA targeted to apurinic/apyrimidinic endonuclease (Ape1), which is a DNA repair enzyme. This siRNA has been used in Dr. Michael Vasko’s laboratory in other studies where it effectively reduced the expression of Ape1 (Vasko et al. 2005). Here, it serves as a negative control. The left lane shows that after treatment with siRNA targeted to Kv1.1, the expression was reduced compared with either the control or Ape1 siRNA-treated neurons. The densitometries are summarized in the bottom panel wherein Kv1.1 siRNA significantly reduced the expression of Kv1.1 by 54% ± 9% (n = 6 separate tissue harvests), whereas the Ape1 siRNA had no effect (n = 2 separate tissue harvests for each condition). In addition, to examine the specificity of the siRNA targeted to Kv1.1, a separate series of experiments determined the expression levels of Kv1.2, a potassium channel a-subunit known to associate with Kv1.1 (see INTRODUCTION), after a 48-h exposure to siRNA targeted to Kv1.1. Analysis of the densitometry showed that the expression level of Kv1.2 (when normalized to the density of actin in each respective lane) was unchanged after siRNA treatment compared with untreated neurons undergoing the same procedure (control Kv1.2/actin 1.0 vs. siRNA-treated 0.99 ± 0.12, n = 3; data not shown). However, when the same gels were probed for Kv1.1, treatment with siRNA targeted to Kv1.1 produced a significant decrease in the expression of Kv1.1 (control Kv1.1/actin 1.0 vs. siRNA-treated 0.59 ± 0.04, n = 3; data not shown). This reduction of 41% was not different from the 54% decrease (P = 0.48, t-test) reported earlier for a separate series of experiments. The lack of effect of siRNA targeted to Ape1 on Kv1.1 expression and the inability of siRNA targeted to Kv1.1 to affect expression of Kv1.2 suggest that siRNA can be used to effectively reduce the expression levels of targeted channels.

**DTX-K suppressed outward potassium currents in untreated but not in siRNA-treated neurons**

To confirm that siRNA targeted to Kv1.1 reduced the functional expression of this channel protein, the inhibitory effects of DTX-K, the antagonist of Kv1.1, on I\textsubscript{K} in untreated control or in neurons exposed to siRNA was examined. Under control conditions, in a representative untreated neuron, the amplitude of the outward I\textsubscript{K} was 4.62 nA measured at +40 mV (Fig. 5A, top left). After a 5-min exposure to 10 nM DTX-K, the amplitude of I\textsubscript{K} decreased to 2.52 nA (top middle of Fig. 5A). These results are summarized in the current–voltage (I–V) relation shown in Fig. 5A (right). Under control conditions, the outward I\textsubscript{K} began to activate at approximately −30 mV. In the untreated neurons, 10 nM DTX-K significantly inhibited the I\textsubscript{K} obtained at +40 mV by 33 ± 6% (control 5.02 ± 1.01 vs. 3.24 ± 1.08 nA, n = 6, paired t-test) after a 5-min exposure (Fig. 5A, right). I\textsubscript{K} was suppressed by 40 ± 9% after 10 min in these same neurons (data not shown). These results indicate that the maximum inhibition was attained after about 5 min and that there was not a longer time-dependent component to the inhibition produced by DTX-K. In the left panel of Fig. 5B, the I\textsubscript{K} sensitive to DTX-K is shown (same neuron in A) and demonstrates that this current exhibits little time-dependent inactivation. The I–V relation for the DTX-K–sensitive I\textsubscript{K} is summarized in the middle panel of Fig. 5B. The DTX-K–sensitive I\textsubscript{K} begins to activate at about −10 mV, which is about 20 mV more depolarized than observed for the total I\textsubscript{K} under different conditions (see Fig. 3).

### Table 1. Effects of DTX-K on membrane properties in untreated and siRNA-treated sensory neurons

<table>
<thead>
<tr>
<th></th>
<th>Number of APs</th>
<th>Firing Threshold, mV</th>
<th>RMP, mV</th>
<th>APD, ms</th>
<th>Rheobase, pA</th>
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<tr>
<td>Untreated, n = 11</td>
<td></td>
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<tr>
<td>Control</td>
<td>2.5 ± 0.3</td>
<td>−10.9 ± 3.0</td>
<td>−57.8 ± 1.9</td>
<td>5.7 ± 0.8</td>
<td>475.6 ± 97.1</td>
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<tr>
<td>DTX-K</td>
<td>7.7 ± 1.5*</td>
<td>−15.1 ± 3.3*</td>
<td>−57.4 ± 1.9</td>
<td>5.4 ± 0.8</td>
<td>231.3 ± 49.3*</td>
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<tr>
<td>siRNA-treated, n = 12</td>
<td></td>
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<tr>
<td>Control</td>
<td>3.0 ± 0.2</td>
<td>−1.6 ± 4.0</td>
<td>−59.7 ± 1.6</td>
<td>7.5 ± 1.3</td>
<td>559.2 ± 17.0</td>
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<tr>
<td>DTX-K</td>
<td>2.4 ± 0.3</td>
<td>−15.5 ± 3.2</td>
<td>−60.8 ± 1.9</td>
<td>8.3 ± 1.4</td>
<td>470.3 ± 134.4</td>
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Values are means ± SE. *P < 0.05, paired t-test. RMP, resting membrane potential; APD, action potential duration.
The capacity of DTX-K to augment excitability is prevented by siRNA treatment

Treatment with siRNA targeted to Kv1.1 lowers the expression of Kv1.1, but functionally what are the consequences of this reduction? To examine this question, the parameters of excitability described earlier were examined in the absence and presence of DTX-K in Kv1.1 siRNA-treated neurons. In a representative Kv1.1 siRNA-treated neuron, the ramp of depolarizing current evoked three APs under control conditions (see Fig. 6A, left). After a 5-min exposure to 10 nM DTX-K (Fig. 6A, right), this neuron fired only two APs, indicating that the excitability was not affected by DTX-K. In a total of 15 sensory neurons, a 5-min exposure to 10 nM DTX-K did not significantly increase the number of APs after treatment with siRNA targeted to Kv1.1 (control 3.1 ± 0.2 vs. 3.5 ± 0.6, P = 0.52, paired t-test). However, in 3 of the 15 neurons, DTX-K caused a twofold increase in the number of APs, suggesting that these particular neurons did not take up the siRNA because the DTX-K–induced increase in the number of APs was quite similar to that observed in the untreated neurons. Based on this, these three neurons were excluded from further analysis. (The values for the number of APs between the control and treated groups for the 15 neurons was not different from the 12 neurons; P = 0.31, ANOVA.) The results obtained from 12 small-diameter capsaicin-sensory neurons are summarized in Fig. 6B. In the Kv1.1 siRNA-treated neurons, a 5-min exposure to DTX-K did not alter the number of evoked APs (control 3.0 ± 0.2 vs. 2.4 ± 0.3 after 5 min, paired t-test). These results indicate that DTX-K was incapable of affecting the excitability after treatment with siRNA targeted to Kv1.1. As with the number of APs, exposure to DTX-K did not change the properties of neurons including the firing threshold, or the rheobase (see Table 1). Similar to the untreated neurons, DTX-K did not affect the resting membrane potential or the duration of the AP (11 of 12 neurons exhibited an AP “hump”) after Kv1.1 siRNA treatment (Table 1, n = 12, paired t-test). Consistent with the idea that siRNA reduced the expression of Kv1.1, in the siRNA-treated neurons, the average value of the firing threshold was close to that measured after a 5-min exposure to DTX-K in the untreated neurons (−16.8 ± 4.0 for siRNA neurons vs. −15.1 ± 3.2 mV for untreated). However, the average value of the rheobase determined in the siRNA-treated neurons was not different from the value obtained for the untreated neurons under control conditions (before DTX-K). These results suggest that after treatment with siRNA targeted to Kv1.1, other potassium channels may assume an important role in setting the rheobase after the loss of Kv1.1. It is also possible that the siRNA targeted to Kv1.1 affected an “off-target” site, which influences the rheobase that we are unaware of presently. In sensory neurons that were exposed to siRNA targeted to Ape1, treatment with DTX-K produced effects that were similar to those observed for untreated neurons. For example, a 5-min exposure to 10 nM DTX-K significantly increased the number of evoked APs (control value 2.3 ± 0.5 vs. after DTX-K 7.8 ± 1.5 APs, n = 4, paired t-test). In these Ape1 siRNA-treated neurons, DTX-K had no effect on the resting membrane potential (control −60.0 ± 3.4 vs. after DTX-K −59.0 ± 3.4 mV, n = 4, paired t-test) or the AP duration (control 5.7 ± 0.8 ms vs. after DTX-K 5.7 ± 1.3 ms, n = 4). Similar results were obtained for those neurons exposed to the transfecting agent, Neuroporter, alone. For example, DTX-K significantly increased the number of APs from a control value of 2.7 ± 0.9 to 6.0 ± 1.2 and decreased the firing threshold by about 10 mV without altering the resting membrane potential (data not shown, n = 3, paired t-test). These observations indicate that Kv1.1 is important in establishing the firing threshold/rheobase of sensory neurons and may be an important target for further modulation of the excitability in sensory neurons.
DISCUSSION

Our results demonstrate that sensory neurons of the DRG express the potassium channel Kv1.1 and that this channel plays an important role in setting the level of excitability. This channel appears to be expressed in neurons of all sizes. Although our observations are not quantitative, they are consistent with previous reports demonstrating that Kv1.1 was present at moderate to high levels (based on an optical density gray-scale calibration) in both small-diameter (<30 μm) and medium- to large-diameter (>30 μm) sensory neurons of the L4/5 DRG of the rat (Ishikawa et al. 1999) and in most neurons (89%) of the nodose ganglia (Glazebrook et al. 2002). These findings are in contrast to those showing that Kv1.1 was detected primarily in large-diameter neurons of the L4–6 DRG (Rasband et al. 2001). The reasons for these differences are not presently clear. In addition, others have reported the expression of the mRNA for Kv1.1 in the L4/5 DRGs using either RT-PCR (Kim et al. 2002) or RNase protection assays (Yang

FIG. 5. DTX-K suppressed $I_K$ in untreated but not in siRNA-treated neurons. A: total $I_K$ recorded from a representative untreated neuron (left) where the maximum current amplitude during the voltage step of $I_K$ measured at +40 mV was 4.62 nA. After a 5-min exposure to 10 nM DTX-K (middle), the amplitude of $I_K$ decreased to 2.52 nA. DTX-K produced a significant decrease in $I_K$ between 0 and 40 mV. Current traces are shown in +20 mV increments for voltage steps between −80 to +40 mV. Lines labeled 0 indicate the zero-current level. The right panel of A summarizes the current–voltage ($I$–$V$) relation before and after a 5-min exposure to DTX-K. B, left: representative traces for the DTX-K–sensitive $I_K$ obtained for the neuron in A. Middle: $I$–$V$ relation for the DTX-K–sensitive $I_K$. Right: comparison of the normalized $I$–$V$ relations for the untreated control $I_K$ and the DTX-K–sensitive $I_K$. C: total $I_K$ from a representative neuron after treatment with Kv1.1 siRNA (left); the amplitude of $I_K$ was 3.67 nA measured at +40 mV. After a 5-min exposure to 10 nM DTX-K (middle), the amplitude of $I_K$ was 3.98 nA. Current traces are shown in +20-mV increments for voltage steps between −80 and +40 mV. Right: summary of the $I$–$V$ relation for the Kv1.1 siRNA-treated sensory neurons before and after a 5-min exposure to 10 nM DTX-K.

FIG. 6. DTX-K did not alter the number of evoked action potentials (APs) after treatment with Kv1.1 siRNA. A, left: representative recording from a Kv1.1 siRNA-treated neuron under control conditions. Right: demonstrates that a 5-min exposure to 10 mM DTX-K did not affect the number of APs evoked by the ramp. B: summary of the effects of DTX-K on the number of APs in 12 siRNA-treated sensory neurons.
from the nodose and trigeminal ganglia (Glazebrook et al. 2002; Stansfeld et al. 1986; Yoshida and Matsumoto 2005) as well as in neurons of the MNTB (Brew and Forsythe 1995; Brew et al. 2003). Furthermore, the notion of Kv1.1 playing a key role in regulating excitability is supported by recordings obtained from mice wherein the gene for Kv1.1 has been deleted. In recordings from pyramidal neurons of the hippocampal CA3 region, the threshold for AP firing resulting from either mossy fiber or antidromic stimulation was reduced and, rather than evoking a single AP as in the wildtype mice, a train of APs was observed in the Kvca1-null mice (Lopantsev et al. 2003; Smart et al. 1998). The resting membrane potentials and the AP durations were not different in the knock-out and the wildtype mice.

We used siRNA to specifically reduce the expression level of this potassium channel. This approach has the advantage over gene deletion in that neurons develop with their normal complement of channels and the target in question is reduced only transiently. Treatment with siRNA targeted to Kv1.1 reduced the protein expression by about 60% and completely removed the inhibition of \( I_K \) produced by DTX-K. It is possible that the remaining 40% of Kv1.1 after siRNA treatment comes from neurons that did not take up the siRNA and/or the detected channel protein was that remaining in intracellular compartments as part of their trafficking (Manganas and Trimmer 2000). In terms of the excitability, after siRNA treatment DTX-K no longer increased the number of APs evoked by the ramp as observed in the untreated, the Neuroporter controls, or the Ape1 siRNA-treated neurons. Thus it seems likely that the Kv1.1-like immunoreactivity detected by the Western blots after siRNA treatment does not represent functional protein. After treatment with Kv1.1 siRNA, several parameters exhibited values that were similar to those determined after exposure to DTX-K in untreated neurons. For example, the firing threshold after siRNA treatment reflected those values measured after exposure to DTX-K, indicating that the transient inhibition produced by DTX-K had similar actions as that caused by reducing the expression level of Kv1.1. In untreated neurons, the rheobase was significantly reduced after DTX-K, although in siRNA-treated neurons the rheobase was similar to that determined for the control value rather than that after DTX-K. The reasons for this are presently unclear. One possibility may involve the heteromeric interactions of Kv1.1 with other potassium channel subunits, such as Kv1.2 or Kv1.6, which may be involved in regulating the rheobase. This notion regarding the contribution of potassium channel heteromultimers is quite complex and clearly requires more detailed investigation.

siRNA has proven to be effective in blocking the actions of other potassium channels (see Gurney and Hunter 2005). In CHO cells, both the expression and current conducted by Kv4.3 was reduced by about 75% after a 48-h treatment with siRNA (Cotella et al. 2005). In HEK 293 cells expressing the two-pore potassium channel TASK-2, siRNA reduced the expression by about 64% and the current measured at pH 7.4 by 40%. Treatment with the scrambled siRNA had no effect. When this siRNA was used in isolated pulmonary artery myocytes, the depolarization produced by a TASK-2-selective inhibitor was reduced significantly compared with the untreated myocytes (Gonczi et al. 2006). These results suggest that siRNA can effectively reduce the expression of ion chan-

J Neurophysiol • VOL 98 • NOVEMBER 2007 • www.jn.org
Kv1.1 AND EXCITABILITY IN SENSORY NEURONS

channels so that their functional aspects can be established in native tissues.

In summary, our results demonstrate that suppression of Kv1.1 by exposure to the selective blocker DTX-K produced an increase in AP firing that was associated with decreases in the firing threshold and the rheobase. Also, siRNA targeted to Kv1.1 reduced the expression of this potassium channel in which the actions of DTX-K to inhibit $I_K$ and enhance AP firing were prevented. Thus dendrotoxin-sensitive potassium channels play an important role in limiting the excitability of sensory neurons. Treatments with siRNA should prove to be effective in establishing the physiological roles of ion channels in excitability where selective antagonists are not yet available.

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