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Received January 21, 2004; accepted March 9, 2004

ABSTRACT
Recent studies revealed that a new compound, KW-7158 [(2S)-(+-)3,3,3-trifluoro-2-hydroxy-2-methyl-N-(5,5,10-trioxo-4,10-dihydrothieno[3,2-c][1]benzothiepin-9-yl)propanamide], can depress the excitability of afferent pathways from the urinary bladder and reduce bladder overactivity induced by chemical irritation of the urinary tract with xylene, an agent that sensitizes capsaicin-sensitive, C-fiber afferent nerves. In the present experiments, we examined the mechanisms that might underlie the depressant effect of KW-7158 on primary afferent neurons by studying the actions of the compound on ion channels and firing in dissociated dorsal root ganglion (DRG) cells from adult rats using whole cell patch-clamp techniques. KW-7158 increased transient, A-type K⁺ currents at concentrations ranging from 50 nM to 1 µM (20–50% increases). Similar effects were seen in fast blue identified bladder afferent neurons. Low concentrations of KW-7158 shortened the action potential duration, produced a 5- to 10-mV hyperpolarization, and inhibited repetitive firing induced by either 4-AP (50 µM) or substance P (0.5 µM) in phasic firing DRG neurons. Above 1 µM, KW-7158 elicited a smaller enhancement of A-type K⁺ currents and in high concentrations inhibited the currents. Tetraethylammonium (5–60 mM) and verapamil (50 µM), which block noninactivating K⁺ currents, did not prevent the facilitatory effects of KW-7158. High concentrations of 4-AP (5 mM) inhibited A-type K⁺ currents and prevented the facilitatory effect of KW-7158 on the remaining currents. These data suggest that KW-7158 enhances A-type K⁺ currents in DRG neurons. Because A-type K⁺ channels regulate afferent neuron excitability and firing properties, KW-7158 is a promising new compound for treatment of hyper-reflexic bladder conditions.

Recent studies in our laboratory (Lu et al., 2002) revealed that a new compound, KW-7158 [(2S)-(++)-3,3,3-trifluoro-2-hydroxy-2-methyl-N-(5,5,10-trioxo-4,10-dihydrothieno[3,2-c][1]benzothiepin-9-yl)propanamide], can depress the excitability of afferent pathways from the urinary bladder and reduce bladder overactivity induced by chemical irritation of the urinary tract with xylene, an agent that activates capsaicin-sensitive, C-fiber afferent nerves. KW-7158 also reduced the rise in blood pressure induced by bladder distension (i.e., the vesico-vascular reflex) in urethane anesthetized rats without altering basal blood pressure (Lu et al., 2002). Systemic capsaicin administration or intravesical application of resiniferatoxin, afferent neurotoxins, produced a similar depression of the vesico-vascular reflex (Cheng et al., 1993). These observations indicate that the vesico-vascular reflex is mediated by C-fiber bladder afferent nerves and provide further support for view that KW-7158 suppresses the activity of bladder afferent nerves.

In the present experiments, we examined the mechanisms that might underlie the depressant effect of KW-7158 on primary afferent neurons by studying the actions of the compound on ion channels and firing in dissociated dorsal root ganglion cells from adult rats using whole cell patch-clamp techniques. Previous patch-clamp studies on bladder afferent neurons demonstrated two types of cells (Yoshimura et al., 1996). The majority (70%) of the neurons were capsaicin-sensitive, C-fiber neurons exhibiting primarily high threshold, tetrodotoxin-resistant Na⁺ channel currents and action potentials (APs), phasic firing (i.e., one or two action potentials) in response to prolonged depolarizing current pulses and low-threshold, fast-inactivating K⁺ currents (A-type cur-

ABBREVIATIONS: KW-7158; (2S)-(++)-3,3,3-trifluoro-2-hydroxy-2-methyl-N-(5,5,10-trioxo-4,10-dihydrothieno[3,2-c][1]benzothiepin-9-yl)propanamide; AP, action potential; DRG, dorsal root ganglion; FB, fast blue; TEA, tetraethylammonium; RP, resting potential; 4-AP, 4-aminopyridine; SP, substance P.
The present studies revealed that KW-7158 enhanced fast-inactivating K\textsuperscript{+} currents and suppressed the firing and electrical excitability of bladder as well as unidentified DRG neurons. These results provide further support for view that bladder dysfunction by suppressing afferent activity and indicate that K\textsuperscript{+} channels in afferent pathways are important targets for pharmacologic treatment of lower urinary tract disorders.

Materials and Methods

Cell Preparation. Potassium currents were recorded in primary cultures of dorsal root ganglion neurons of adult male rats using the whole cell patch-clamp technique. Cells where isolated as described previously (Sculptoreanu et al., 1995). Freshly dissected ganglia (T6-L1) were suspended in DMEM containing 10% heat inactivated horse serum and 5% fetal bovine serum (Sigma-Aldrich, St. Louis, MO), and plated on collagen-coated 35-mm petri dishes (Biocoat; Collaborative Research, Bedford, MA). Neurons were plated at 25000–30000/dish. Primary cultures were kept in a 95% air, 5% CO\textsubscript{2} incubator at 37°C. The population of DRG neurons that innervate the urinary bladder were labeled by retrograde axonal transport of fluorescent dyes and dye-labeled neurons were identified using an inverted phase contrast microscope (Nikon, Tokyo, Japan) with fluorescent attachments (UV-1A filter; excitation wavelength, 365 nm). In patch-clamp recordings in FB-labeled bladder afferent neurons, after recording action potential characteristics and capsaicin sensitivity (1 μM), the external solution was changed to the solution containing 150 mM choline-Cl, 5 mM CaCl\textsubscript{2}, 2.5 mM MgCl\textsubscript{2}, 10 mM HEPES, pH adjusted to 7.4 with HCl. Use of phosphate buffer allowed for simultaneous recording of Na\textsuperscript{+}, K\textsuperscript{+} currents, membrane potentials, and AP firing in the same cells. The pipette (intracellular) solution was high K\textsuperscript{+} solution (Gold et al., 1996). Activation of K\textsuperscript{+} currents was fitted to the Boltzmann equation I = I\textsubscript{max} \cdot \exp(-(V - V\textsubscript{1/2})/k) / (1 + \exp(-(V - V\textsubscript{1/2})/k)) + constant, where V\textsubscript{1/2} is the half-activation voltage and k, slope factors of curve 1 and 2. This made the implicit assumption that currents were roughly divided into low- and high-threshold components (Gold et al., 1996). Activation curves for K\textsuperscript{+} currents were plotted as normalized G/K\textsubscript{max} versus test voltages, where the G\textsubscript{K} (conductance) was determined by dividing the current in the Boltzmann relationship by the driving force [G\textsubscript{K} = I/(V - E\textsubscript{K})]. The reversal potential, E\textsubscript{K}, was calculated assuming a purely K\textsuperscript{+}-permeable channel. Inactivation of peak K\textsuperscript{+} currents, before and after verapamil and KW-7158, was fitted with a sum of two Boltzmann curves [I = I\textsubscript{1}/(1 + \exp(-(V - V\textsubscript{1/2,1})/k1)) + (I\textsubscript{2}/(1 + \exp(-(V - V\textsubscript{1/2,2})/k2)) constant; terms defined as for activation). Data were plotted as I\textsubscript{0}/I\textsubscript{Kmax} versus prepulse voltages used to generate inactivation curves (constant driving force). Membrane potential and action potential generation in response to rectangular pulse current injections were measured in the same cells after switching voltage-clamp to current-clamp mode. Extracellularly applied drugs were pipetted from stock solutions at 10 to 100 times the final concentration and rapidly mixed in the recording chamber as described previously (Sculptoreanu et al., 1995). Results are reported as mean ± S.E.M. Statistical analysis used t test, two-tailed, and unequal variance. Data were considered not statistically different (N.S.) if p > 0.05.

Pharmacological Materials. The extracellular solution in these experiments was either Dulbecco’s phosphate buffer (Sigma-Aldrich) or reduced Na\textsuperscript{+} (65 mM)-high TEA (60 mM) Na\textsuperscript{+} buffer of the following composition: 65 mM NaCl, 5 mM Na\textsubscript{2}ATP, 6 mM TEA-Cl, 4 mM KCl, 5 mM CaCl\textsubscript{2}, 2.5 mM MgCl\textsubscript{2}, 10 mM HEPES, pH adjusted to 7.4 with HCl. Use of phosphate buffer allowed for simultaneous recording of Na\textsuperscript{+}, K\textsuperscript{+} currents, membrane potentials, and AP firing in the same cells. The pipette (intracellular) solution was high K\textsuperscript{+} (140 mM), which contained 120 mM KCl, 10 mM KH\textsubscript{2}PO\textsubscript{4}, 2 mM MgCl\textsubscript{2}, 10 mM EGTA, 10 mM HEPES, pH adjusted to 7.4 with HCl. To this solution, 3 mM Mg-ATP, 0.3 mM CAMP, and 0.5 mM Tris-GTP were added just before doing the experiments. In those experiments, in which bladder DRG neurons were labeled by retrograde axonal transport of fast blue (Yoshimura and de Groat, 1997, 1999), dyes-labeled neurons were identified using an inverted phase contrast microscope (Nikon, Tokyo, Japan) with fluorescent attachments (UV-1A filter; excitation wavelength, 365 nm). In patch-clamp recordings in FB-labeled bladder afferent neurons, after recording action potential characteristics and capsaicin sensitivity (1 μM), the external solution was changed to the solution containing 150 mM choline-Cl, 5 mM KOH, 0.03 mM CaCl\textsubscript{2}, 10 mM HEPES, 3 mM Mg(OH)\textsubscript{2}, and 10 mM D-glucose, adjusted to pH 7.4 with HCl, and K\textsuperscript{+} currents were recorded. KW-7158, which was provided by Kyowa Hakko Kogyo Co., Ltd. (Tokyo, Japan) has been patented for therapeutic use in treatment of urinary incontinence and bladder hyperactivity (Yoshida et al., 1998; Yamagata et al., 2002). KW-7158 at doses of 0.01 and 0.1 mg/kg (oral administration) inhibits the premicturition contractions (nonvoiding contraction) in rats with spinal cord injury (Yamagata et al., 2002). KW-7158 at doses of 0.01 and 0.1 mg/kg (oral administration) was 16.7 ± 2.0 ng/ml (approximately 412 nM), respectively (n = 4; unpublished data). Here, we tested similar KW-7158 concentrations ranging from 50 nM to 1 μM. The concentration dependence of the KW-7158 effect in patch-clamp experiments was done in a cumulative manner with at most four compound concentrations tested on each individual neuron.

Results

KW-7158 Hyperpolarized Small Diameter DRG Cells and Facilitated Transient Outward Currents Responsible for AP Repolarization. KW-7158 was tested on small


Facilitatory Effect of KW-7158 on K⁺ Currents. In another series of experiments, different holding potentials and K⁺ channel blockers (i.e., verapamil; Catacuzzo et al., 1999; 50 μM; n = 10) or TEA (Gold et al., 1996; 10–60 mM; n = 26; Fig. 2) were used to examine the effect of KW-7158 on specific types of K⁺ currents. In some of these experiments, the activation protocol consisted of a rectangular pulse (800 ms in duration) to +60 mV, from a holding potential of −90 mV (Fig. 1C). This was followed by a 0.8-s depolarizing prepulse to −40 mV, which partially inactivated A-type K⁺ currents, and then a second depolarizing pulse to +60 mV.
similar to the first in the sequence (Fig. 1C; n = 12 cells tested). In these experiments, the A-type K⁺ currents activated by the second test pulse in the sequence were only partially inactivated (70–80%), and KW-7158 increased the currents before (5–50% enhancements) and after (5–20%) the −40-mV interpulse (Fig. 1, C and D). Subsequently, we used a 0.8-s duration depolarization interpulse to −20 mV, to produce a more complete inactivation of most of the A-type K⁺ currents induced by the second test pulse to +60 mV (Fig. 2). This stimulus protocol prevented facilitatory effects of KW-7158 on the noninactivating outward currents generated by the second test pulse (Fig. 3A).

The current densities of A-type K⁺ currents obtained by subtracting the delayed rectifier currents after the −20-mV interpulse from the total currents activated from a holding potential of −90 mV (Fig. 3) were 25.4 ± 3.1 pA/pF in control versus 25.1 ± 1.6 pA/pF (p > 0.05) after (−)verapamil (50 μM). These currents were reduced (22.8 ± 4.5 pA/pF; p < 0.05) in the presence of 20 mM TEA (8% reduction from control) and reduced further in the presence of 60 mM TEA.

To determine the contribution of A-type K⁺ currents to the total currents, the changes in peak amplitude of K⁺ current in response to a square pulse to +60 mV, 800 ms in duration, at each compound concentration were subtracted from changes in currents to an identical pulse after a 0.8-s depolarization prepulse to −20 mV. A, change in peak amplitude of K⁺ currents in control experiments before (○) and after (●) inactivation of A-type K⁺ currents. B, changes in A-type K⁺ currents in control currents (○) and after partial block of delayed rectifier K⁺ currents with 20 mM TEA (●). C, changes in A-type K⁺ currents in control currents (○) and in the presence of 50 mM (−)verapamil (△). D, changes in A-type K⁺ currents in control currents (○) and in the presence of 60 mM TEA (■).

Fig. 2. Effect of delayed rectifier K⁺ channel blockers on K⁺ currents and KW-7158 effect in DRG neurons. The voltage protocol consisted of a square pulse to +60 mV, 800 ms in duration, from a holding potential of −90 mV. This was followed by a 0.8-s depolarization prepulse to −20 mV, applied immediately after a short, 50-ms repolarization to −60 mV. Except for the interpulse interval to −20 mV, the time course of the stimulus was identical to that shown in Fig. 1E. This interpulse inactivated most of the A-type K⁺ currents activated by a second depolarizing pulse to +60 mV identical to the first in the sequence. A, (−)verapamil (50 μM) inhibited the K⁺ currents activated during the interpulse depolarization to −20 and most of the delayed outward current activated by the second test pulse to +60 mV. B, KW-7158 (0.5 μM) enhanced the rapidly inactivating A-type currents but had no effect on the verapamil-insensitive noninactivating currents activated during the second test pulse. C, TEA (20 mM) inhibited the K⁺ currents activated during the interpulse depolarization to −20 mV and most of the delayed outward current activated by the second test pulse to +60 mV. D, K⁺ currents recorded in high TEA (60 mM) solutions. KW-7158 had no effect on the noninactivating delayed rectifier channels, which were TEA-insensitive (0.5 μM).
(20.5 \pm 3.1 \text{ pA/pF}; 20\% \text{ reduction}; p < 0.01). (-)-Verapamil (50 \mu M) inhibited 19.6 \pm 2\% of the total K⁺ currents activated during the first pulse from −90 mV, inhibited >90\% of the currents activated during interpulse depolarization to −20 mV and inhibited by 73.1 \pm 1.7\% the noninactivating outward currents activated by the second test pulse to +60 mV (Figs. 2, A and B, and 3C; n = 10; p < 0.001). KW-7158 (0.05–1.0 \mu M) enhanced the rapidly inactivating A-type K⁺ currents (5–50\% increases) but had no effect on the verapamil-insensitive nonactivating residual currents activated during the second test pulse (Fig. 2B).

TEA (20 mM) inhibited 47.7 \pm 0.8\% of the K⁺ currents activated during the first pulse, >90\% of the currents activated during the interpulse depolarization to −20 mV and 58.4 \pm 1.1\% of the noninactivating outward current activated by the second test pulse to +60 mV. (Fig. 2C; n = 26; p < 0.001). Higher TEA concentrations (60 mM) inhibited 60.4 \pm 2.8 and 82.4 \pm 1.6\% of the currents during the first and second test pulse, respectively. (Figs. 2D and 3D; n = 13; p < 0.01). A-type K⁺ currents elicited from a holding potential of −90 mV, in the presence of 20 mM TEA (Fig. 3B) or 60 mM TEA (Figs. 2D and 3D), were enhanced (5–48\% increases) by KW-7158 (0.05–1.0 \mu M) to magnitudes similar to those seen in control experiments. In a separate series of experiments, we determined that high concentrations of 4-aminopyridine (4-AP; 5 mM), which blocked the majority (>90\%) of the A-type K⁺ currents in DRG neurons (Gold et al., 1996), and significantly prolonged the AP duration (5–20\% increases), eliminated the facilitatory effect of either 0.5 \mu M (n = 5) or 1 \mu M KW-7158 (n = 8).

In untreated cells, K⁺ currents were roughly divided into inactivating (A-type) and noninactivating currents (delayed rectifier). The amplitude of the total outward currents elicited by depolarizing pulses to +60 mV were 72 \pm 4 \text{ pA/pF} (n = 28) at a holding potential of −90 mV, and 48.0 \pm 2.4 \text{ pA/pF} after an 800-ms duration prepulse to −20 mV. The noninactivating K⁺ currents comprised between 40 and 80\% (65 \pm 4\%) of the total K⁺ currents (Figs. 2 and 3A) and were 4-AP insensitive (n = 12). In the absence of TEA or verapamil, KW-7158 increased the total K⁺ currents (before prepulse) at concentrations ranging from 0.05 to 4 \mu M (20–50\% maximal enhancements of currents; Fig. 3A), but it had no effect on nonactivating currents (after prepulse). Above 1 \mu M, KW-7158 progressively reduced the enhancement of the inactivating K⁺ currents and inhibited both the inactivating (before prepulse; Fig. 3A, empty circles) and noninactivating (after prepulse; Fig. 3A, filled circles) K⁺ currents. At 5 to 500 \mu M, these inhibitory effects were concentration dependent and could exceed 60\% inhibition of the total currents.

Treatment with TEA or (-)-verapamil did not prevent either the facilitatory effects of low concentrations of KW-7158 or the inhibitory effects of higher KW-7158 concentrations (Fig. 3). However, in the presence of TEA, the concentration dependence of the KW-7158 inhibitory effects were shifted to higher compound concentrations, and this shift was further increased after changing from 20 to 60 mM TEA (Fig. 3, B and D). The inhibitory effects of KW-7158 were not mimicked by similar concentrations of the vehicle used to prepare the KW-7158 stock solution (dimethyl sulfoxide, \leq0.01\%; n = 8 cells tested; not shown).

To determine the kinetics and voltage dependence of activation and inactivation of K⁺ currents, a combined two-pulse activation-inactivation protocol was used, consisting of a series of rectangular pulses from a holding potential of −80 mV. In addition to voltage separation of inactivating and noninactivating currents, we used verapamil to block nonactivating currents before the prepulse. We presumed that unlike TEA, which blocks certain inactivating K⁺ currents (Gold et al., 1996), verapamil would more selectively inhibit the noninactivating, delayed rectifier currents (Catacuzzeno et al., 1999). The prepulse, 1021.5 ms in duration, ranging from −130 to +90 mV, was used to generate K⁺ current activation and the inactivation. The inactivation curve was measured after a brief, 24.5-ms interpulse at −80 mV and a second test pulse to +60 mV, 249.5 ms in duration (Fig. 4). The interpulse of 24.5 ms was determined in separate experiments to be brief enough for insignificant recovery from inactivation of K⁺ currents. Figure 4 shows the average current densities for the activation in control (empty circles) and after addition of (-)-verapamil (50 \mu M, empty squares) and KW-7158 (0.5 \mu M, empty triangles) in that sequence. The K⁺ currents activated near −50 mV and reached near maximal amplitudes at +90 mV (Fig. 4A). The fit of activation curves with a sum of two Boltzmann equations (Fig. 4A), revealed before addition of drugs two components of activation having amplitudes of 42 \pm 7 \text{ pA/pF} (A1; V1 \alpha \approx −52 \pm 2 \text{ mV}) and 19 \pm 3 \text{ pA/pF} (A2; V2 \alpha \approx −0.13 \pm 0.5 \text{ mV}), respectively. Addition of (-)-verapamil (50 \mu M) blocked the majority of noninactivating K⁺ currents (Fig. 4B) as reported by Catacuzzeno et al. (1999). This inhibition was accompanied by a positive shift in the voltage dependence of activation of both the low- and high-threshold currents and a positive shift in the voltage dependence of inactivation of the high threshold currents (Table 1). The enhancement of K⁺ channel currents by KW-7158 after block of noninactivating currents with (-)-verapamil did not change either the voltage dependence of activation nor the voltage dependence of inactivation, except for a 10-mV negative shift in the voltage dependence of inactivation of both the high and low-threshold currents (Fig. 4; Table 1). KW-7158 after (-)-verapamil increased both the low threshold (79\% increase to 34 \pm 5 \text{ pA/pF}; p < 0.01) and high-threshold components (33\% increase to 24 \pm 4 \text{ pA/pF}; p < 0.01).

In another series of experiments, the effects of KW-7158 on K⁺ currents were examined in FB-labeled bladder afferent neurons obtained from the L6-S1 DRG. Slowly inactivating A-type K⁺ currents were isolated by subtraction of outward K⁺ currents activated by depolarizing pulses to +60 mV from a holding potential of −40 mV from those activated from a holding potential of −120 mV as described previously (Yoshimura and de Groat, 1999). The activity of KW-7158 (1 \mu M) was tested on 1) inactivating A-type K⁺ currents; 2) a mixture of partially inactivated A-type K⁺ currents and noninactivating delayed rectifier K⁺ currents, in bladder afferent neurons that were sensitive to capsaicin and therefore presumably C-fiber afferent neurons. KW-7158 (1.0 \mu M) increased the inactivating A-type K⁺ currents that were activated at the −120-mV holding potential (26 \pm 3\% increase; n = 6), but had a smaller effect (10 \pm 3\% increase; n = 6) on partially inactivated A-type K⁺ currents elicited from a holding potential of −40 mV.

**KW-7158 Reversal of the Repetitive Firing Induced by Substance P (SP) or 4-AP.** Another series of experiments tested the effect of KW-7158 on the tonic firing in-
duced by prolonged depolarizing current pulses in phasic neurons treated with either 4-AP (50 μM) or SP (0.5 μM). As shown in Fig. 5, smaller diameter DRG cells exhibited phasic firing in response to 600-ms duration, 80- to 200-pA amplitude, depolarizing current pulses. The firing consisted of a small number of action potentials (range 1–4; mean 1.7 ± 0.3 APs; Fig. 5, A, B, E, and F; n = 25). Larger diameter neurons exhibited tonic firing to the same stimulus (>5 APs; mean 8.4 ± 1.4 APs; n = 7; Fig. 5, C and D). In phasic neurons, SP lowered the threshold of AP firing by 7 mV from −25 ± 1 mV before compound application to −31 ± 2 mV (n = 9; p < 0.01) and by 4 mV in tonic firing DRG neurons (−36 ± 2 mV; control, −41 ± 2 mV after SP; p < 0.05; Fig. 5A). In phasic neurons, 4-AP lowered the threshold by 5 mV to −30 ± 1 mV (n = 5; p < 0.01; Fig. 5E). In phasic neurons, SP (Fig. 5, A and B) or 4-AP (50 μM; Fig. 5, E and F) also significantly increased the number of APs induced by the depolarizing current pulse (318% increase after SP; n = 9). In untreated DRG neurons, KW-7158 (0.5 μM) had modest (3.5-mV reduction) but statistically insignificant effect on the threshold of AP firing in phasic neurons (−21.5 ± 2.5 mV; n = 12; p > 0.05), and no measurable effect on the threshold of APs in tonic neurons (n = 7; not shown). However, KW-7158 reversed the effect of SP (Fig. 5, A and B) on firing in phasic neurons and increased the threshold of AP firing by 8 mV to levels similar to those seen before SP application (−23 ± 2 mV; n = 5; p = 0.3). KW-7158 also reversed the effect of 4-AP (50 μM; Fig. 5, E and F) on firing in phasic neurons. The effect of KW-7158 on SP facilitation of firing could be antagonized by administration of 4-AP (50 μM; Fig. 5, A and B).

In tonically firing neurons that generated five or more APs (8.4 ± 1.4 APs; n = 7; Fig. 5, C and D), SP (50 μM) increased the number of APs (149% increase; n = 7) and KW-7158 decreased this effect (50% decrease; Fig. 5, C and D).
TABLE 1

Parameters of Boltzmann fits of K⁺ current activation and inactivation in control experiments (before drugs) and after addition of (−) verapamil (50 μM) and KW-7158 (0.5 μM) added in that sequence in the same cells

<table>
<thead>
<tr>
<th></th>
<th>Control (−) Verapamil</th>
<th>KW-7158</th>
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<tbody>
<tr>
<td><strong>Low-threshold currents (1st Boltzmann)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Activation</td>
<td></td>
<td></td>
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<tr>
<td>( P )</td>
<td>0.20 ± 0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>( V_{1/2} )</td>
<td>−52 ± 2</td>
<td>−38 ± 6</td>
</tr>
<tr>
<td>( k )</td>
<td>6.4 ± 2.2</td>
<td>6.2 ± 0.9</td>
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<tr>
<td>Inactivation</td>
<td></td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>( P_{\text{max}} )</td>
<td>0.04 ± 0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>( V_{1/2} )</td>
<td>−110 ± 12</td>
<td>−58 ± 2</td>
</tr>
<tr>
<td>( k )</td>
<td>8 ± 1</td>
<td>27 ± 1</td>
</tr>
<tr>
<td><strong>High-threshold currents (2nd Boltzmann)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Activation</td>
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<td></td>
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<tr>
<td>( P_{\text{max}} )</td>
<td>0.80 ± 0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>( V_{1/2} )</td>
<td>−0.13 ± 0.5</td>
<td>10.7 ± 5.9</td>
</tr>
<tr>
<td>( k )</td>
<td>21 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>Inactivation</td>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td>( P_{\text{max}} )</td>
<td>0.17 ± 0.04</td>
<td>0.37 ± 0.06</td>
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<tr>
<td>( V_{1/2} )</td>
<td>−0.5 ± 0.5</td>
<td>0.001</td>
</tr>
<tr>
<td>( k )</td>
<td>13 ± 2</td>
<td>6 ± 2</td>
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</table>

Data for fits of average conductance normalized to \( G_{\text{max}} \) before drugs (activation) and current densities normalized to \( I_{\text{max}} \) before drugs in eight neurons. Parameters for Boltzmann fits of K⁺ current activation and inactivation were determined as explained under Materials and Methods and Results. The smooth curves shown in Fig. 4, A and B were fitted with these values. Data are averages; S.E.M.; \( t \) test relative to values in control experiments, before drugs, two-tailed, unequal variance, level of significance: ns, not significant; \( p < 0.01 \), or \( p < 0.001 \).

The resting potential in the phasic neurons (\( \alpha \)) was on the average 165% larger in the tonic neurons (\( \beta \)). The remaining currents are noninactivating, delayed rectifier types (Gold et al., 1996; Ishikawa et al., 1999). The transient K⁺ currents are all sensitive 4-AP, but only one (Kv1.4; Pongs, 1992) is also sensitive to TEA blockade (Gold et al., 1996). Our data in Figs. 2 and 3 suggest that after blockade of delayed rectifier K⁺ channels with TEA or (−)verapamil, KW-7158 enhanced a rapidly inactivating current without having an effect on residual, TEA-verapamil-insensitive nonactivating K⁺ currents. The facilitatory effects of low concentrations of KW-7158 (0.05–1.0 μM) were selective for 4-AP-sensitive channels because 5 μM 4-AP, which blocked a large fraction of transient K⁺ currents, also prevented the enhancement by low concentrations of KW-7158.

Higher concentrations of KW-7158 presumably inhibited both inactivating currents and a number of nonactivating currents nonselectively because inhibition of these currents by (−)verapamil or TEA reduced the inhibitory effects of KW-7158 and shifted the concentration dependence of inhibition to higher concentrations. It is also important to note that after (−)verapamil blockade of nonactivating currents; KW-7158 facilitated both low-threshold and high-threshold inactivating currents without significant shifts in either the voltage dependence of activation or inactivation (Fig. 4). At high concentrations, KW-7158 inhibits >50% of the currents after prepulse (Fig. 3A, filled circles). Verapamil blocks >80% of the nonactivating currents (Fig. 2B). Therefore, we think that most of the KW-7158 inhibition of verapamil-sensitive K⁺ currents after the −20-mV prepulse in Fig. 3A (filled circles) is due to inhibition of nonactivating currents.

One possible interpretation of TEA-induced shift in the concentration dependence of K⁺ channel inhibition by high KW-7158 concentrations is that KW-7158 may act on multiple A-type K⁺ currents, which have different affinities for the compound, only some of which are TEA-sensitive, i.e., Kv1.4 (Ishikawa et al., 1999). An alternative interpretation is that KW-7158 and TEA may act allosterically on the same subtype of channel, the result being the observed shift in the concentration dependence of KW-7158 response. Indeed, verapamil, a more selective blocker of delayed rectifier K⁺ channels in both chick DRG neurons (Trequattrini et al., 1998; Catacuzzeno et al., 1999) and mammalian ganglion

Discussion

The present studies revealed that KW-7158 enhanced transient K⁺ currents and suppressed the firing in bladder as well as unidentified DRG neurons in the rat. KW-7158 also hyperpolarized the membrane potential (−5–10 mV) and reduced the AP duration (5–80%). KW-7158 had no effect on Na⁺ currents, AP upstroke, or overshoot of AP. These observations provide support for the conclusions from previous in vivo experiments that KW-7158 reduced urinary bladder hyperactivity by suppressing the afferent limb of the micturition reflex pathway (Lu et al., 2002).

KW-7158 Facilitates a Transient, 4-AP-Sensitive K⁺ Channel. A-type K⁺ currents are important determinants of neuronal firing (Locke and Nerbonne, 1997; Erisir et al., 1999; Tkatch et al., 2000). Rat DRG neurons express at least six subtypes of K⁺ currents (Gold et al., 1996). Three of these currents, exhibit rapid to slow rates of inactivation (A-type) and correspond to Kv4 subtypes (Pongs, 1992; Tkatch et al., 2000), Kv1.2 and Kv1.4 types of K⁺ channels (Ishikawa et al., 1999). The remaining currents are noninactivating, delayed rectifier types (Gold et al., 1996; Ishikawa et al., 1999). The transient K⁺ currents are all sensitive 4-AP, but only one (Kv1.4; Pongs, 1992) is also sensitive to TEA blockade (Gold et al., 1996). Our data in Figs. 2 and 3 suggest that after blockade of delayed rectifier K⁺ channels with TEA or (−)verapamil, KW-7158 enhanced a rapidly inactivating current without having an effect on residual, TEA-verapamil-insensitive nonactivating K⁺ currents. The facilitatory effects of low concentrations of KW-7158 (0.05–1.0 μM) were selective for 4-AP-sensitive channels because 5 μM 4-AP, which blocked a large fraction of transient K⁺ currents, also prevented the enhancement by low concentrations of KW-7158.

Higher concentrations of KW-7158 presumably inhibited both inactivating currents and a number of nonactivating currents nonselectively because inhibition of these currents by (−)verapamil or TEA reduced the inhibitory effects of KW-7158 and shifted the concentration dependence of inhibition to higher concentrations. It is also important to note that after (−)verapamil blockade of nonactivating currents, KW-7158 facilitated both low-threshold and high-threshold inactivating currents without significant shifts in either the voltage dependence of activation or inactivation (Fig. 4). At high concentrations, KW-7158 inhibits >50% of the currents after prepulse (Fig. 3A, filled circles). Verapamil blocks >80% of the nonactivating currents (Fig. 2B). Therefore, we think that most of the KW-7158 inhibition of verapamil-sensitive K⁺ currents after the −20-mV prepulse in Fig. 3A (filled circles) is due to inhibition of nonactivating currents.

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neurons (Hogg et al., 1999), also shifted the inhibition to higher KW-7158 concentrations (Fig. 3). However, the use-dependent nature of verapamil block of noninactivating currents (Hogg et al., 1999; Fig. 2A) prevented a more detailed analysis of the actions of inhibitory concentrations of KW-7158 in the presence of verapamil. On the other hand, neither TEA nor (-)-verapamil, which nonselectively block K+ currents, prevented the facilitatory effects of low concentra-

![Figure 5](image_url)

**Fig. 5.** Effect of KW-7158 (KW; 0.5 μM) on frequency of firing increased by either SP (0.5 μM) or 4-AP (50 μM) in phasic neurons or SP in tonic neurons. A, recording of APs in a phasically firing dorsal root ganglion neuron, which fired at most three APs in response to depolarizing current injections. Bath application of SP increased the number of APs to eight. This increase was reversed by KW-7158. Addition of 4-AP in the presence of SP and KW-7158 led to an increase in firing to five APs. B, average number of APs (n = 9 neurons) for experiments such as shown in A. In phasic neurons (1–4 APs in response to an 80–200-pA, 600-ms duration depolarizing injection), SP produced a significant (p < 0.001) increase in the number of APs. Addition of KW-7158 reversed the effect of KW (N.S., not significant from the control before addition of drugs; p = 0.5). After reversal of SP effect by KW-7158, 4AP increased the number of APs in all neurons tested (p < 0.001). The RP in these cells was −51 ± 2 mV, and the Cm was 45 ± 4 pF. C, recording of action potentials in a tonically firing dorsal root ganglion neuron, which fired seven APs in response to depolarizing current injections. Bath application of SP increased the number of APs to 11. This increase was reversed by KW-7158. Addition of 4-AP in the presence of SP and KW-7158 led to an increase in firing to 10 APs. D, average number of APs for experiments such as shown in C in seven neurons. In tonic neurons (5–15 APs in response to an 80–200-pA, 600-ms duration depolarizing injection), SP produced a significant, 149% increase in firing (p < 0.01). Addition of KW-7158 reversed the effect of SP (p = 0.3). After reversal of SP effect by KW-7158, 4-AP produced a 131% increase in firing in all neurons tested (p < 0.001). The RP in these cells was −53 ± 2 mV, and the Cm was 71 ± 14 pF. E, recording of APs in a phasically firing dorsal root ganglion neuron, which fired one AP in response to depolarizing current injections. Bath application of 4-AP increased the firing to four APs. This increase was reversed by KW-7158. F, average number of APs for experiments such as shown in E in 16 neurons. In these experiments, 4-AP produced a significant (p < 0.001) increase in firing. Addition of KW-7158 reversed the effect of 4-AP. The RP in these cells was −54 ± 2 mV, and the Cm was 42 ± 2 pF. G, stimulus protocol for current-clamp experiments shown in A, C, and E. With membrane potential held at −65 mV, a short 5-ms current injection (80–200 pA) was used to trigger a single action potential, a second pulse 600 ms in duration was applied 300 ms after the first in the sequence.
tions of KW-7158, but unmasked larger facilitatory effects at higher concentrations of KW-7158 (Fig. 3). Among the inactivating K⁺ currents known to be expressed in DRG neurons, only Kv1 subtypes are sensitive to TEA blockade (Wissmann et al., 2003), thus the most likely target for the KW-7158 facilitatory effect is a Kv4 K⁺ channel subtype (Pongs, 1992; Tkatch et al., 2000).

**KW-7158 Reduces Hyperexcitability Induced by SP or 4-AP.** SP and neurokinin A released from DRG neurons act on receptors expressed inafferent neurons and afferent nerve terminals in the periphery and activate phospholipase C (Saban et al., 1997; Ruggieri, 1998). Disruption of the preprotachycyphin gene, which codes for SP, leads to an impaired response to chemical irritation of the urinary tract in mice (Kiss et al., 2001). Activation of PKC phosphorylates and inhibits a number of K⁺ channels (Hoffman and Johnston, 1998; Zhang et al., 2001) to promote hyperactivity in DRG neurons. Therefore, an autoregulatory mechanism mediated by neurokinin receptors and protein kinase C may contribute to acute nociceptive sensitization in these neurons. KW-7158 had no effect on the threshold for AP firing in phasic neurons, or the threshold and AP firing in tonic neurons, consistent with our previous observations that KW-7158 had no effect on basal bladder activity (Lu et al., 2002). However, KW-7158 inhibited both the SP and 4-AP-induced hyperactivity in phasic neurons and SP-enhanced activity in tonic neurons (Fig. 5), suggesting that this compound may act to suppress both the SP- and 4-AP-promoted nociceptive sensitization in phasic neurons and mechanosensitivity in tonic DRG neurons (Djouhri et al., 1998; Lawson, 2002). These effects extend to identified bladder neurons consistent with our observations demonstrating inhibition of hyperactive bladder activity (Lu et al., 2002).

**KW-7158, a Potential Suppressor of Visceral Hyper-sensitivity.** Nociceptive afferent neurons have slowly conducting axons in the C and Aδ range and have longer duration AP and long-lasting afterhyperpolarizing potentials, and lower maximal firing rates (Lawson, 2002). Larger, nonnociceptive neurons have myelinated, more rapidly conducting AβPs, and fire short-duration APs tonically at high frequency (Lawson, 2002). In small diameter DRG neurons, and capsaicin-sensitive bladder neurons, KW-7158 (0.05–1.0 μM) enhanced the rapidly inactivating A-type K⁺ currents (5–50% increases) but had no facilitatory effect on the verapamil-TEA-sensitive, nonactivating currents activated after an 800-ms depolarization to −20 mV (Fig. 3). These facilitatory effects were presumably responsible for the effects of KW-7158 on resting potential, excitability (Fig. 5) and AP shape (Fig. 1). Therefore, it seems likely that KW-7158 opens a K⁺ channel, which activates near the resting membrane potential and controls the repolarization phase of AP in phasic, small diameter DRG neurons. It is interesting that KW-7158 also increased excitability in tonically firing neurons (Fig. 5), which were presumably Aδ firing neurons. This observation suggests that KW-7158 may also facilitate rapidly inactivating K⁺ channels expressed in larger neurons.

**KW-7158, a Potential Inhibitor of Chronic Nociceptive Hyperactivity.** Recently, K⁺ channels have received considerable attention as targets for the treatment of a variety of disorders (Shieh et al., 2000). In this study, we showed that a novel K⁺ channel agonist, KW-7158, inhibited repetitive activity induced by SP or 4-AP in phasic small- to medium-sized (<50 pF) DRG neurons. In certain forms of abnormal excitability of nociceptive neurons, such as cyclophosphamide-induced cystitis in urinary bladder (Yoshimura and de Groat, 1999), or axotomy (Kim et al., 1998), A-type K⁺ channel behavior is altered. KW-7158 would be expected to also lower the firing rates, increase the threshold for initiation of AP, and have beneficial effects in this condition, by lowering the hyperexcitability.

It seems likely that KW-7158, which decreases the hyperexcitability induced acutely in normal DRG neurons by either SP or 4-AP, by increasing the threshold for AP generation, would also lower excitability in afferents sensitized by chronic inflammation. In a recent study of cyclophosphamide-induced cystitis, there was a significant decrease in the current densities of an A-type K⁺ current (Yoshimura and de Groat, 1999). Therefore, it seems reasonable to conclude that KW-7158, by increasing A-type K⁺ currents, would be a useful therapeutic agent for both acute and chronic conditions involving nociceptive sensitization of afferent neurons.

K⁺ channels are also expressed as heterologously assembled multimers (Isacoff et al., 1990; Ruppersberg et al., 1990; Sheng et al., 1993; Wang et al., 1993) with other regulatory protein subunits (Kubista et al., 1999; Schrader et al., 2002; Wang et al., 2002). K⁺ channel openers that may be selective for ATP-sensitive (Hu and Kim, 1997) or other subtypes of K⁺ channels (Rundfeldt, 1997; Rasband et al., 2001; Lu et al., 2002) are envisioned as useful therapeutic agents in a variety of neurological disorders. Our data does not establish whether KW-7158 acts on the channel or an auxiliary subunit, but it clearly establishes that the current activated by the compound is a transient, rapidly inactivating current. A clear resolution of these questions will require more direct molecular biological approaches. Regardless of its exact mechanism of action, KW-7158 seems to belong to a novel class of drugs that enhance A-type K⁺ channel activities. Because A-type K⁺ channels play such a key role in neuronal firing, KW-7158 is a promising agent for treatment of a number of disorders that are associated with afferent neuron hyperexcitability, including urinary bladder dysfunction induced by spinal cord injury.

**References**


Kiss et al. (1997).

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