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Dihydropyridine block of voltage-dependent K⁺ currents in rat DRG neurons

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Abstract

The dihydropyridines nifedipine, nimodipine and Bay K 8644 are widely used as pharmacological tools to assess the contribution of L-type voltage-gated Ca²⁺ channels to a variety of neuronal processes including synaptic transmission, excitability and second messenger signaling. These compounds are still used in neuronal preparations despite evidence from cardiac tissue and heterologous expression systems that they block several voltage-gated K⁺ (Kv) channels. Both because these compounds have been used to assess the relative contribution of L-type Ca²⁺ channels to several different processes in dorsal root ganglion (DRG) neurons and because a relatively wide variety of Kv channels present in other neuronal populations are present in DRG neurons, we determined the extent to which dihydropyridines block Kv currents in these neurons. Standard whole cell patch clamp techniques were used to study acutely dissociated adult rat DRG neurons. All three dihydropyridines tested blocked Kv currents in DRG neurons; IC₅₀ values for nifedipine and nimodipine-induced block of sustained Kv currents were 14.5 μM and 6.6 μM, respectively. The magnitude of sustained current block was 44 ± 1.6%, 60 ± 2%, and 56 ± 2.9% with 10 μM nifedipine, nimodipine and Bay K 8644, respectively. Current block was occluded by neither 4-aminopyridine (5 mM) nor tetraethylammonium (135 mM). Dihydropyridine-induced block of Kv currents was not associated with a shift in the voltage-dependence of current activation or inactivation, the recovery from inactivation, or voltage dependent block. However, there was a small use-dependence to the dihydropyridine-induced block. Our results suggest that several types of Kv channels in DRG neurons are blocked by mechanisms distinct from those underlying block of Kv channels in cardiac myocytes. Importantly, our results suggest that if investigators wish to explore the contribution of L-type Ca²⁺ channels to neuronal function, they should consider alternative strategies for the manipulation of these channels than the use of dihydropyridines.

Keywords

Sensory neuron; off-target effects; high-threshold voltage dependent Ca²⁺ channel; voltage-clamp

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INTRODUCTION

The dihydropyridines such as nifedipine and nimodipine were originally developed for the treatment of angina and hypertension because of their potent vasodilatory properties (Vater et al., 1972, Leonard and Talbert, 1982). While it was originally appreciated that block of Ca^{2+} entry into coronary smooth muscle was the primary mechanism of action (Henry, 1980, Sorkin et al., 1985), investigators subsequently realized that these compounds were selective for a subpopulation of high threshold voltage-gated Ca^{2+} channels (VGCC). These were referred to as L-type channels because they underlie a long-lasting current in response to membrane depolarization. Biophysical (Tsien and Nilius, 1987), pharmacological (Bean, 1989) and ultimately molecular biological (Catterall et al., 2005) data confirmed both the existence of multiple types of VGCC and the utility of nifedipine and nimodipine as pharmacological tools with which to study L-type channels. The potency of these compounds with reported IC_{50} values in the range of 100-300 nM in both cardiac (Bean, 1989) and neuronal (Catterall et al., 2005) tissue adds to the appeal of these compounds as pharmacological tools. Furthermore, the discovery Bay K 8644, a dihydropyridine capable of increasing L-type channel open probability, meant that it was possible to assess L-type channel function with selective channel activators and blockers. Consequently, nifedipine, nimodipine and/or Bay K 8644 have been widely used in neurophysiological experiments designed to assess the relative contribution of L-type channels to processes ranging from transmitter release (Sabria et al., 1995, Sugiura and Ko, 1997, Rosato Siri and Uchitel, 1999) to transcriptional regulation (Brosenitsch et al., 1998, Brosenitsch and Katz, 2001, Toescu et al., 2004, D'Ascenzo et al., 2006).

However, there is evidence that in addition to their activity at L-type channels, dihydropyridines also block voltage gated Na^{+} (Yatani and Brown, 1985), Ca^{2+} -dependent K^{+} channels (Klockner and Isenberg, 1989), and voltage gated K^{+} (Kv) channels (Gotoh et al., 1991, Lin et al., 2001, Gao et al., 2005) in cardiac and vascular smooth muscle cells. There is also evidence that these compounds block Kv channels in invertebrate neurons (Nerbonne and Gurney, 1987). However, the impact of dihydropyridines on Kv channels in vertebrate neurons remains to be studied in detail as available evidence from embryonic preparations is conflicting. In one study, Kv channels in vertebrate neurons appeared to be dihydropyridine-resistant (Nerbonne and Gurney, 1987) while in two additional studies, Kv channels appeared to be dihydropyridine-sensitive (Valmier et al., 1991, Fagni et al., 1994).

Both because of a cell geometry that is amenable to clamp control (i.e., a sphere devoid of processes) with the standard whole cell patch configuration in voltage-clamp, and the fact that they express a wide variety of voltage-gated channels present in other neuronal populations (Gold, 2006), the acutely isolated dorsal root ganglion (DRG) neuron is widely used as a model system to study the properties of neuronal voltage-gated channels (Gold, 2001). These neurons are also widely studied because of their importance in all forms of somatosensation, including nociception (Gold and Caterina, 2008). Not surprisingly, dihydropyridines have been used to assess the contribution of L-type channels in DRG neurons to processes critical to somatosensation including neurotransmitter release (Perney et al., 1986, Rane et al., 1987, White, 1996), transcriptional regulation (Ai et al., 1998, Brosenitsch et al., 1998), and cell signaling (Usachev and Thayer, 1999, Chaban et al., 2003). Given that an "off-target" effect such as a block of a Kv channel would impact the interpretation of results obtained from such studies, the present study was conducted to determine the extent to which Kv channels in a vertebrate neuron are sensitive to nifedipine, nimodipine and Bay K 8644. Our results indicated that all three compounds block Kv currents in DRG neurons in a manner distinct from that described in cardiac and smooth muscle cells.

EXPERIMENTAL PROCEDURES

Adult (200-250g) male Sprague-Dawley rats were used for this study. Rats were housed in the University of Pittsburgh animal facility in groups of three on a 12:12 light dark schedule. Food and water were available *ad lib*. All the experiments were approved by the University of Pittsburgh Animal Care and Use Committee and performed in accordance with NIH guidelines as well as guidelines established by the International Association of the Study of Pain for the use of laboratory animals in research. All efforts were employed in order to minimize the number of animals used in this study.

DRG neuron dissociation

DRG neurons were harvested, enzymatically treated, mechanically dissociated and plated on laminin and ornithine coated glass cover-slips as previously described (Lu et al., 2006). All neurons were studied between 3 to 9 h after removal from the animal.

Electrophysiology

Standard voltage-clamp techniques in the whole cell patch configuration were used to record Kv currents in isolated DRG neurons with a HEKA EPC10 (HEKA Electronik, Lambrecht/Pfaff, Germany) amplifier controlled with Pulse software (v 8.65). Cells were continuously perfused with a bath designed to isolate Kv currents from other currents in DRG neurons. This solution contained (in mM): Choline-Cl 135; KCl 5; CoCl₂ 2.5; MgCl₂ 1.2; 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) 5; and glucose 10; pH was adjusted to 7.4 with Tris-Base, and osmolality was adjusted to 320 mOsm with sucrose. CoCl₂ was used instead of CaCl₂ to minimize activation of Ca²⁺ dependent conductances. Patch electrodes pulled from borosilicate glass pipettes (1.5 mm o.d., WPI Sarasota Springs, FL) had a resistance of 1.5-2.0 MΩ when filled with electrode solution that contained: (in mM): KCl 30; K-methanesulfonate (MES) 110; Na-MES 5; MgCl₂ 1; HEPES 10; Ethylene glycol-bis(2-aminoethylether)-N,N,N',N'-tetraacetic acid (EGTA) 5; ATP-Mg 2; GTP 1; pH was adjusted to 7.2 with Tris-base, and osmolality was adjusted to 310 mOsm with sucrose.

After formation of a tight seal (>1GΩ) and compensation of pipette capacitance with amplifier circuitry, whole cell access was obtained by rupturing the membrane. Cell capacitance was determined with five hyperpolarizing pulses (10 ms) from -60 to -80 mV. Whole cell capacitance and series resistance were compensated with the amplifier circuitry with series resistance compensation > 80%. Neurons were held at -60 mV. Current-voltage (I-V) relationship for total Kv current was determined with a series of 400 ms voltage steps from -90 to +60 mV in a 10 mV increment with a 5 second interval. To determine reversal potential of Kv current, a tail current protocol was employed before and after application of nifedipine or nimodipine. This protocol consisted of a 100 ms voltage step to +40 mV to activate Kv current, followed by a 50 ms step to voltages ranging between -100 and -30 mV. Kv current was evoked with a 425 ms voltage step to +40 mV from -100 mV every 10 sec to observe the time dependence of dihydropyridine block. A conventional double pulse protocol was used to assess the impact of dihydropyridines on steady-state inactivation, where the pre-pulse duration was 500 ms. The presence of use-dependent block was assessed with a series of 50 ms pulses to +40 mV applied at 1, 5, 10 and 20Hz. Finally, recovery from inactivation was assessed with a two-pulse protocol consisting of a 500 ms conditioning step to -30 mV and a test step to +40 mV separated by an incrementally increasing voltage step to -100 mV. All experiments were performed at room temperature.

Data analysis

Voltage dependence of current (I) activation was determined from conductance (G)-voltage (V) curves derived from I-V data, where $G = I$ normalized by driving force (i.e., the difference

between command potential (V_m) and reversal potential (V_{rev}), where V_{rev} was directly measured). G-V curves were fitted with a Boltzmann equation of the form: $G = G_{max}/(1 + \exp[(V_{1/2} - V_m)/k])$, where G = observed conductance, G_{max} = the fitted maximal conductance, $V_{1/2}$ = the potential for half activation or availability, V_m = command potential and k = the first slope factor. Nifedipine and nimodipine concentration-response data were fitted with a modified Hill equation of the form: Fractional inhibition ($I_{drug}/I_{baseline}$) = $[MAX_{inhib}/(Drug + IC_{50})]^n$ where MAX_{inhib} = maximal fractional inhibition; Drug = concentration of nifedipine or nimodipine; IC_{50} = half-maximal inhibitory concentration; n = Hill coefficient. Inactivation data were fitted with a modified Boltzmann equation of the form: $I = (I_{max} - (I_{max} * a))/(1 + \exp((V_m - V_{1/2})/k)) + I_{max} * a$, where I = observed current, I_{max} = fitted maximal current, $V_{1/2}$ = the potential for half inactivation, V_m = command potential, k = slope factor and a = the fraction of noninactivated current. Recovery from inactivation rates were determined by fitting data with a double exponential equation of the form: Fractional recovery = $F1 * (1 - \exp(-t_{rec}/\tau_1)) + (1 - F1) * (1 - \exp(-t_{rec}/\tau_2))$, where $F1$ is the fraction of current recovered with the first time constant, τ_1 is the first time constant, t_{rec} is the voltage-step duration between conditioning and test commands, and τ_2 is the second time constant. A paired t-test was used to determine whether the influence of dihydropyridines were statistically significant. Repeated measure ANOVA was used to assess the voltage-dependence of the block of Kv currents. $P < 0.05$ was considered statistically significant.

Drugs

Nifedipine, nimodipine, and Bay K 8644 (Sigma St Louis, MO, USA) were dissolved in dimethyl sulfoxide (DMSO) (Sigma) stored as a 100mM stock solution in dark at -20°C , and diluted in bath solution immediately prior to use. Dihydropyridine containing solutions were protected from light in all experiments. The highest concentration of DMSO was 0.1%, a concentration that had no detectable effect on Kv currents in our experiments.

RESULTS

Nifedipine, nimodipine and Bay K 8644 block Kv current in DRG neurons

To facilitate clamp control, Kv current was recorded in small to medium diameter (i.e., 25-32 μm) DRG neurons. Since 10 μM is a concentration of dihydropyridine frequently used in neurophysiological studies, we first determined the effect of this concentration on total Kv current. All three compounds significantly attenuated Kv current. An example of dihydropyridine-induced block of Kv current is shown in Fig. 1. Currents appeared to decay more rapidly in the presence of nifedipine (Fig 1B); the time constant of current decay was reduced from 250 ± 28.5 to 165 ± 11.4 ms, $n = 15$, $p < 0.01$. The nifedipine sensitive current was relatively rapidly activating and slowing inactivating (Fig 1C). Current block was reversible with $> 90\%$ recovery within 5 minutes after removing the dihydropyridine from the bath solution (Fig. 1D, $n = 3$). The reversal potential for the nifedipine sensitive current (measured from tail current amplitudes as described in methods) was -63 ± 1.9 mV ($n=4$), which was not significantly different from that observed for currents evoked in the presence (-60 ± 0.47 mV) or absence of nifedipine (-62 ± 1.3 mV), and while relatively depolarized to that predicted for K^+ by the Nernst Equation with the electrophysiological solutions used, these values are consistent with those previously obtained from Kv currents in DRG neurons (Gold et al., 1996) and appear to reflect the less than perfect selectivity of K^+ channels in sensory neurons. The presence of 10 μM nifedipine had no significant influence on the voltage-dependence of current activation (Fig 1E and Table 1, $n = 15$, $p > 0.05$). Similar results were obtained with nimodipine and the L-type channel “activator” Bay K 8644 (data not shown). Pooled data from neurons studied with all three compounds indicate that 10 μM , block of sustained (i.e., current at the end of a 400 ms voltage step) current was significantly ($p < 0.01$) larger than that of peak current (Fig 1F).

Concentration-dependent block of Kv current by nifedipine and nimodipine

Concentration-response data were collected to further characterize both the potency and efficacy of dihydropyridine-induced block of Kv currents. Nifedipine and nimodipine were sequentially applied at increasing concentrations from 1 to 100 μM (Fig 2). The time course of inhibition was monitored for each neuron with a voltage step to +40 mV every 10 seconds. Each concentration was applied for 60 seconds, and the fraction of current block as determined with the current evoked immediately prior to the application of the next higher concentration. Block of sustained Kv current was relatively more complete than that of peak current for both nifedipine (Fig 2A) and nimodipine (Fig 2C). Cumulative concentration-response data for both compounds indicated that sustained currents were blocked with a small, but significantly greater potency than that of peak currents (Fig 2 B and D): IC_{50} of nifedipine were $14.5 \pm 0.9 \mu\text{M}$ and $24.7 \pm 2.5 \mu\text{M}$ ($n = 7$) for sustained and peak currents respectively, while IC_{50} of nimodipine were $6.6 \pm 0.8 \mu\text{M}$ and $16.6 \pm 1.7 \mu\text{M}$ ($n = 5$) for sustained and peak currents respectively. This difference in potency appeared to reflect the presence of a rapidly inactivating Kv current in most neurons that was relatively resistant to dihydropyridine-induced block. The Hill coefficients for block of both peak and sustained currents were close to 1 (i.e., 1.03 ± 0.06 and 1.18 ± 0.08 for nifedipine induced block of peak and sustained currents, respectively).

Dihydropyridine-induced block of Kv currents are occluded by neither TEA or 4-AP

Multiple types of Kv currents have been described in DRG neurons (Gold et al., 1996). And while concentration-response data suggested that block of Kv currents in DRG was virtually complete, differences in the potency of dihydropyridine-induced block of peak and sustained currents raised the possibility that Kv currents in DRG neurons are differentially sensitive to dihydropyridines. To further explore this possibility, we assessed the impact of nimodipine (10 μM and 100 μM) in the presence of saturating concentrations of the A-type channel blocker 4-aminopyridine (4-AP, 5 mM, $n = 10$) or the delayed rectifier-type channel blocker tetraethylammonium (TEA, 135 mM, $n = 7$). For the TEA experiments, choline-Cl was replaced with TEA-Cl. While there is evidence that TEA can block inactivating Kv channels (Colinas et al., 2008) and 4-AP can block delayed rectifier type channels (Bordey and Sontheimer, 1999), these compounds can differentiate broad classes of Kv currents in DRG neurons (Gold et al., 1996). Consistent with the suggestion that these compounds block largely non-overlapping populations of channels in DRG neurons, TEA blocked $53 \pm 7\%$ of peak and $76 \pm 4\%$ of sustained Kv current ($n = 7$) while 4-AP blocked $78 \pm 3\%$ of peak and $51 \pm 4\%$ of sustained Kv current ($n = 9$). Consistent with our concentration-response data, 4-AP resistant currents were blocked with an IC_{50} lower than 10 μM (Fig 3A), while block of a slowly inactivating TEA-resistant current was greater than 10 μM (Fig 3B).

Mechanisms of dihydropyridine-induced block of Kv currents

Studies of cloned Kv channels from heart cells (Kv4.3) indicated that dihydropyridine-induced decrease in current reflected an increase in the fraction of inactivated channels as a result of a dihydropyridine-induced leftward shift in the inactivation curve (Hatano et al., 2003, Bett et al., 2006) and/or a decrease in the rate of recovery from inactivation (Hatano et al., 2003). While the -100 mV prepulse employed prior to evoking Kv currents in DRG neurons should minimize the impact of dihydropyridines on the available current, we sought to determine whether a similar increase in inactivation increased the block of Kv currents in DRG neurons. Steady-state inactivation and recovery from inactivation data were collected before and after the application of dihydropyridine (10 μM). Neither nifedipine ($n = 7$) nor nimodipine ($n = 5$) had a detectable influence on the voltage-dependence ($V_{1/2}$ or slope) of steady-state inactivation (Fig 4, Table 1). However, nimodipine did produce a significant ($p < 0.05$) decrease in the fraction of non-inactivatable current (Table 1). Similarly, nifedipine (10 μM , $n = 5$) had

no detectable influence on the recovery from inactivation (Fig 4, Table 1). To pool recovery data from different neurons, current evoked with each test pulse was normalized to that evoked with a test pulse to +40 following a 500 ms pre-pulse to -100 mV. Pooled data are plotted as fractional recovery versus the recovery pulse duration (Fig 4). τ_1 was 73.1 ± 2.2 ms in the absence of nifedipine and was 78.9 ± 7.8 ms in the presence of $10 \mu\text{M}$ nifedipine ($p > 0.05$).

Use dependent block

There is evidence that dihydropyridine-induced block of cardiac Kv channels is use-dependent (Bett et al., 2006). To determine whether block of neuronal Kv channels exhibits similar properties, Kv current was evoked at 1, 5, 10 and 20 Hz before and after the application of nifedipine ($10 \mu\text{M}$); Figure 5A and 5B are data obtained at 1 and 10 Hz, respectively. The fraction of current evoked on the 20th pulse (P20) relative to that evoked on the first pulse (P1) was used as a measure of use-dependent block. There was a small use-dependent attenuation of peak and steady-state Kv current evoked at 1, 5, 10 and 20 Hz in the absence of nifedipine. Use-dependent inhibition data were analyzed with a mixed design repeated measures ANOVA to assess the presence of significant effects associated with nifedipine and frequency of stimulation as well as an interaction between the two. There were statistically significant ($p \leq 0.01$) influences of both factors (Fig 5C). Importantly, the fractional increase in use-dependent block in the presence of nifedipine was small and therefore unlikely to have a large impact on the magnitude of the off-target effects of dihydropyridines. There was no statistically significant interaction between the frequency of stimulation and the presence of nifedipine.

Voltage dependence of block

While a binding site responsible for dihydropyridine-induced block of Kv channels has yet to be identified, data from several studies of cardiac Kv channels suggested that the block has voltage-dependence, such that the magnitude of channel block is inversely related to the extent of channel activation (Zhang et al., 1997, Missan et al., 2003, Gao et al., 2005). To assess the presence of voltage-dependence to the block of Kv currents in DRG neurons, we analyzed I-V data collected before and after application of nifedipine. Pooled data normalized to peak outward current at +60 mV suggested little, if any voltage-dependence to the block of either peak (Fig 6A) or sustained (Fig. 6B) Kv currents in DRG neurons. Similar results were obtained with nimodipine ($n = 6$, data not shown).

DISCUSSION

There were three main observations in our study. First, the majority of Kv current in DRG neurons is blocked by the dihydropyridines, nifedipine, nimodipine, and Bay K 8644. Second, the magnitude of this block is slightly increased in a use-dependent manner. Third, dihydropyridine-induced block of Kv channels in DRG neurons is not associated with a shift in the voltage-dependence of inactivation, the rate of inactivation, the rate of recovery from inactivation, or voltage-dependence.

Dihydropyridine-induced block of Kv channels at concentrations necessary to block VGCC

The most important implication of our results arises from the observation that there is an overlap between the concentration range of dihydropyridine needed to block L-type Ca^{2+} channels and that which is able to block Kv channels. In preliminary experiments on DRG neurons, we were able to confirm that $10 \mu\text{M}$ nifedipine is close to a saturating concentration for Ca^{2+} channel block and that in 4 neurons 300 nM nifedipine blocked $57 \pm 5\%$ of the Ca^{2+} current blocked by $10 \mu\text{M}$ nifedipine. This suggests an IC_{50} for nifedipine-induced block of L-type channels in DRG neurons similar to that reported for the block of L-type channels in cardiac cells which ranged between 200 nM and $1 \mu\text{M}$ (Gurney et al., 1985, Charnet et al., 1987, Mecca and Love, 1992, Diocot et al., 1995). Data from heterologous expression studies with the 4 alpha subunits

underlying L-type channels suggests that variability in dihydropyridine sensitivity reflects properties of the underlying channels where IC_{50} values of 139 nM, 3 μ M and 300 nM have been reported for the block of $CaV_{1.2-1.4}$, respectively (Catterall et al., 2005). Thus, with an IC_{50} of ~ 10 μ M for block of Kv, complete block of all but $CaV_{1.2}$ channels will require concentrations of dihydropyridine that may also block a significant fraction of Kv current.

Kv channel subtypes sensitive to dihydropyridine-induced block

Data from heterologous expression studies indicate that dihydropyridine sensitivity depends on Kv subunit composition. For example, nifedipine blocked channels composed of homomeric Kv1.3 or Kv3.1 with IC_{50} of values of 5 μ M and 131 μ M, respectively (Grissmer et al., 1994). Consistent with the suggestion that subunit composition influences dihydropyridine sensitivity, nifedipine blocked A-type and rapidly activating delayed rectifier type currents in human atrial myocytes with IC_{50} values of 26.8 μ M and 8.2 μ M, respectively (Gao et al., 2005). There may be species differences as well, however, in light of data from guinea-pig heart suggesting that considerably higher concentrations of nifedipine are needed to block delayed rectifier type currents with IC_{50} values between 275 μ M and 360 μ M (Zhabyeyev et al., 2000). Despite the presence of at least 6 distinct Kv current in DRG neurons (Gold et al., 1996) which reflect the presence of a number of Kv subunits (Rasband et al., 1998, Rasband et al., 2001, Chi and Nicol, 2007), our data from rat DRG neurons are generally consistent with those from human cardiac myocytes that the potency of dihydropyridine-induced block of TEA-sensitive delayed rectifier type currents was slightly greater than that of 4-AP sensitive A-type currents. That said, a relatively insensitive rapidly inactivating A-type current was clearly present in the majority of neurons studied (i.e., see Figure 2). Importantly, because both A-type and delayed rectifier type of Kv current are involved in the regulation of sensory neuron excitability (Yoshimura and de Groat, 1999, Harriott et al., 2006) and delayed rectifier type currents influence action potential duration and therefore Ca^{2+} entry and transmitter release (Werz and MacDonald, 1983), dihydropyridine-induced block of Kv channels will counter the influence of dihydropyridine-induced block of VGCC in excitability and transmitter release in sensory neurons.

Mechanisms of block

Open channel block—Dihydropyridine-induced block of several Kv channels studied in heterologous expression systems, including Kv4.3 (Bett et al., 2006), Shaker (Kv1) family subunits (Avdonin et al., 1997) such as hKv1.5 channel (Zhang et al., 1997), involves open-channel block. Such a blocking mechanism is readily manifest as an increase in both the extent and rate of current decay during prolonged channel activation. And while an increase in the rate of current decay was observed with 10 μ M nifedipine in the present study (the time constant of current decay was reduced from 250 ± 28.5 to 165 ± 11.4 ms, $p < 0.01$), we suggest this change is not due to open channel block of Kv channels in sensory neurons. This suggestion is based on the observations that 1) there are multiple Kv currents in sensory neurons, 2) delayed rectifier type currents were relatively more sensitive to dihydropyridine-induced block than A-type currents, 3) there was no change in the rate of current decay when nifedipine was applied in the presence of TEA, and 4) use-dependent block detected with a repeated pulse protocol was small. Thus, the apparent increase in the rate of current decay observed in the presence of 10 μ M nifedipine is likely to reflect the selective loss of delayed rectifier type of current, leaving a larger fraction of inactivating current to account for the apparent increase. Nevertheless, because there is evidence that several Shaker family (Kv1) subunits are present in sensory neurons (Chi and Nicol, 2007) in addition to Kv4.3 (Chien et al., 2007), our results suggest that if channels composed of these subunits are functional in sensory neurons, they are blocked by a mechanism(s) distinct from that described in heterologously expressed channels.

Influence on voltage dependent activation, inactivation and recovery from inactivation—

There is also evidence that dihydropyridine-induced block of Kv channels, in particular, those composed of Kv4.3, may involve an increase in channel inactivation as a result of a hyperpolarizing shift in the voltage-dependence of inactivation and/or a decrease in the rate of recovery from inactivation (Calmels et al., 2001, Hatano et al., 2003, Bett et al., 2006). Despite evidence that Kv4.3 is present in DRG neurons (Chien et al., 2007), we failed to detect changes in activation, inactivation or recovery from inactivation. The fact that others have also failed to detect similar dihydropyridine-induced changes in channel gating in cardiac myocytes (Gao et al., 2005), suggests that subunit composition influences the mechanism of dihydropyridine-induced block of Kv channels, where Kv4.3 in sensory neurons and cardiac myocytes may be present in heteromultimers (Colinas et al., 2008). Alternatively, as suggested above, the processing of Kv channels in native tissues may be distinct from that in heterologous expression systems.

Voltage dependence of channel block—As with open channel block and channel gating, data from previous studies suggest dihydropyridine-induced block of Kv channels may also have voltage-dependence such that the magnitude of channel block is inversely proportional to the magnitude of the voltage-command used to evoke Kv currents. This phenomena has been reported for both native (Missan et al., 2003) and heterologously expressed (Zhang X 1997, Avdonin V and Hoshi T J Gen Physiol 1997) cardiac Kv channels. The pattern of voltage-dependent block described in cardiac Kv channels is consistent with an open channel block, whereby the blocker is “knocked-off” its binding site in the channel pore by K^+ leaving the cell under an ever increasing driving force. That there was little evidence of a voltage-dependent block of Kv currents in DRG neurons is also consistent with the suggestion that the mechanism of dihydropyridine-induced block of Kv currents in DRG neurons involves a mechanism(s) distinct from that of Kv channels in cardiac tissue.

Summary and Conclusions

In summary, we have clearly demonstrated that the dihydropyridines nifedipine, nimodipine and Bay K 8644 block Kv currents in sensory neurons with a potency that has considerable overlap with that of dihydropyridine-induced block of L-type Ca^{2+} channels present in the nervous system. Block of Kv currents in sensory neurons appears to involve mechanisms distinct from those described for the block of Kv channels present in cardiac tissue. Importantly, the possibility that a significant fraction of available Kv channels were blocked in previous analyses of L-type channel function means that estimates of the relative contribution of these Ca^{2+} channels are incorrect. Errors may be over- or under-estimates, depending on the systems studied. For example, a dihydropyridine-induced block of Kv currents in a study designed to estimate the contribution of L-type channels to the activation of BK channels would result in an over-estimate of L-type channel function. Conversely, in studies designed to assess the contribution of L-type channels to synaptic transmission, block of Kv channels would counter the influence of a block of Ca^{2+} channels, resulting in an underestimate of L-type channel function. Furthermore, the simultaneous block of L-type and Kv channels, when only block of L-type channels was anticipated can account for a number of paradoxical observations in the literature. For example, the frequency of spontaneous excitatory post-synaptic currents (sEPSC) in spinal laminae I and II increased after 10 μ M nimodipine application to block L-type channels (Bao et al., 1998) and transmitter release increased at neonatal rat neuromuscular junction after 10 μ M nifedipine (Sugiura and Ko, 1997, Rosato Siri and Uchitel, 1999). Given that all dihydropyridines have comparable effects on cardiac Kv currents, it is likely that results obtained with nifedipine, nimodipine and Bay K 8644 in DRG neurons can be extended to other members of this family of compounds, suggesting that a different class of drug should be used in subsequent analyses of L-type channel function.

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List of abbreviations

4-AP	4-aminopyridine
DRG	dorsal root ganglion
IC₅₀	concentration resulting in an inhibition that is 50% of maximum
K_v	voltage-gated K ⁺
TEA	

tetraethylammonium

VGCC

voltage-gated Ca^{2+} channel

Figure 1.

Dihydropyridines inhibit Kv currents in DRG neurons. Current was evoked with 400 ms voltage steps at 10 mV increments between -90 mV and +60 mV, following a 500 ms pre-pulse to -100 mV. **A.** Current evoked prior to the application of nifedipine (baseline) was stable. **B.** There was a significant reduction in Kv current following the application of nifedipine (10 μM). **C.** The nifedipine sensitive current was obtained by digitally subtracting current evoked in the presence of nifedipine from the current evoked in its absence. **D.** Current evoked at +60 mV, from the DRG neuron shown in A, before (baseline) and after (Nif. 10 μM) the application of nifedipine, and again 5 min after removing nifedipine from the bath (Washout). **E.** The voltage-dependence of Kv activation was similar in the presence and absence of nifedipine (10 μM). G-V data from each neuron (n = 15) were pooled following normalizing with the maximal conductance (Gmax) determined before and after application of nifedipine. **F.** The mean fractional decrease (calculated as $(I_{\text{baseline}} - I_{\text{drug}}) / I_{\text{baseline}}$) in peak evoked current was significantly ($p < 0.01$) smaller than that of sustained current (taken at the end of the 400 ms depolarizing voltage step) for nifedipine (n = 15), nimodipine (n = 9) and Bay K 8644 (n = 5). All pooled data are expressed as Mean $\pm$ s.e.m.

Figure 2.

Concentration-dependent inhibition of total Kv by nifedipine and nimodipine. **A.** Current was evoked from a DRG neuron at +40 mV at 0.1 Hz before (baseline) and then after the application of subsequently higher concentrations (indicated) of nifedipine. **B.** Fractional inhibition of peak and sustained Kv current from 7 DRG neurons were fitted with a modified Hill equation; IC_{50} values 23.8 μ M and 14.7 μ M for peak and sustained current respectively. **C.** An example of another DRG neuron studied in described in A, before and after application of nimodipine. **D.** Pooled nimodipine data ($n = 5$) yielded IC_{50} values of 16.6 and 6.1 μ M for peak and sustained current, respectively.

Figure 3.

A. Kv currents were evoked in DRG neurons as described in Fig 1, in the presence of 4-aminopyridine (4-AP, 5 mM) before (a) and after 10 μM (b) and 100 μM (c) nimodipine. Pooled data ($n = 10$) indicate that close to 70% of 4-AP resistant current is blocked by 10 μM nimodipine while over 90% is blocked by 100 μM nimodipine (d). Because peak and sustained currents in the presence of 4-AP are comparable, only pooled data from sustained currents are plotted. **B.** Kv currents were evoked in DRG neurons in the presence of tetraethylammonium (TEA) before (a) and after application of 10 μM (b) and 100 μM (c) nimodipine. Pooled data ($n = 7$) confirm that peak TEA resistant current is considerably less sensitive to nimodipine than 4-AP resistance current (d).

Figure 4.

Double-pulse protocols were used to assess steady-state inactivation (**A**) and recovery from inactivation (**B**) of DRG neurons before (**A** and **B**, Baseline) and after (**C** and **D**, Nif. 10 μ M) the application of nifedipine. A 500 ms inactivating prepulse was used to assess steady-state inactivation while a 500 ms voltage step to -30 mV was used to induce inactivation from which recovery was assessed with voltage steps to -100 mV. **E**. Pooled steady-state inactivation data ($n = 7$) were normalized with respect to I_{max} . **F**. Pooled recovery from inactivation data ($n = 7$) were normalized with respect to peak outward current evoked with a test pulse to +40 mV.

Figure 5.

Use-dependent block of Kv currents evoked at +40 mV was assessed at stimulation frequencies of 1, 5, 10 and 20 Hz. Pooled data from 1 Hz (**A**, $n = 14$) and 10 Hz (**B**, $n = 7$) before and after the application of nifedipine (10 μ M) are plotted. **C**. Data for each neuron were analyzed as a fractional decrease (P_{20} / P_1) and pooled. The change in fractional decrease as a function of frequency of stimulation was significant ($p < 0.01$) as was the influence of nifedipine (*, $p = 0.01$). There was no interaction between the two factors.

Figure 6.

To assess the presence of voltage-dependence block of Kv current by nifedipine (Nif.) I-V data for Peak (**A**) and sustained (**B**) currents in the absence (Baseline) and presence of 10 μ M and 100 μ M Nifedipine were normalized to that evoked at +60 mV at baseline, and pooled ($n = 15$).

Parameters were obtained from steady-state inactivation data fitted with a modified Boltzmann equation ($n = 7$) slope, $V_{1/2}$ and fraction of non-inactivating currents (a) as well as recovery from inactivation ($n = 7$) data fitted with a double exponential equation used to obtain the fraction of current recovered over the first time constant ($F(\tau_1)$) and the first (τ_1) and second (τ_2) time constant of recovery. τ_2 should be considered an estimate because the values obtained were close to the longest recovery pulse employed (30 s). A paired t-test was used to assess the impact of nifedipine (10 μ M) or nimodipine (10 μ M) on each parameter. Cells were left empty for data not collected.

Table 1

Parameter	Peak		Sustained		Peak		Sustained	
	Baseline	Nifedipine	Baseline	Nifedipine	Baseline	Nimodipine	Baseline	Nimodipine
Slope (mV)	7.6 $\pm$ 2.4	8.5 $\pm$ 1.3	8.0 $\pm$ 0.8	8.9 $\pm$ 1.7	9.0 $\pm$ 2.8	16.9 $\pm$ 7.2	9.1 $\pm$ 2.6	12.6 $\pm$ 4.4
$V_{1/2}$ (mV)	-57.8 $\pm$ 2.4	-59.7 $\pm$ 2.5	-57.5 $\pm$ 2.7	-58.2 $\pm$ 3.9	-63.3 $\pm$ 5.4	-60.6 $\pm$ 2.1	-62.7 $\pm$ 5.2	-69.0 $\pm$ 4.6
a	0.32 $\pm$ 0.05	0.3 $\pm$ 0.05	0.44 $\pm$ 0.06	0.51 $\pm$ 0.05	0.53 $\pm$ 0.12	0.23 $\pm$ 0.01*	0.57 $\pm$ 0.11	0.50 $\pm$ 0.01
$F(\tau_1)$	0.44 $\pm$ 0.09	0.47 $\pm$ 0.08						
τ_1 (ms)	84.2 $\pm$ 9.3	111.6 $\pm$ 22.7						
τ_2 (s)	26 $\pm$ 6	20 $\pm$ 4						

* $p < 0.05$