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Dihydropyridine inhibition of neuronal calcium current and substance P release*

Stanley G. Rane, George G. Holz IV, and Kathleen Dunlap

Department of Physiology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA

Abstract

Dihydropyridine (DHP) calcium channel antagonists, which inhibit the slowly inactivating or L-type cardiac calcium (Ca) current, have been shown to be ineffective in blocking ^{45}Ca influx and Ca-dependent secretion in a number of neuronal preparations. In the studies reported here, however, the antagonist DHP nifedipine inhibited both the L-type Ca current and potassium-evoked substance P (SP) release from embryonic chick dorsal root ganglion (DRG) neurons. These results suggest that, in DRG neurons, Ca entry through L-type channels is critical to the control of secretion. The inhibition of Ca current by nifedipine was both voltage and time-dependent, significant effects being observed only on currents evoked from relatively positive holding potentials maintained for several seconds. As expected from these results, nifedipine failed to inhibit L-type Ca current underlying the brief plateau phase of the action potential generated from the cell's normal resting potential; likewise, no significant effect of the drug was observed on action potential-stimulated SP release evoked by electrical field stimulation. The results of this work are discussed in terms of an assessment of the role of L-type Ca channels in neurosecretion.

Keywords

Dorsal root ganglion neurons; Calcium current; Transmitter release; Dihydropyridines; Nifedipine; Patch clamp

Introduction

Dihydropyridine calcium (Ca) channel antagonists bind to high-affinity sites on cardiac and smooth muscle and they also block the depolarization-induced entry of Ca into these tissues (for review see Miller and Freedman 1984). This blockade of Ca influx is due to the selective interaction of the dihydropyridines (DHPs) with voltage-dependent Ca channels. Despite electrophysiological similarities between neuronal Ca channels and the DHP-sensitive Ca channels in cardiac and smooth muscle, a number of studies have shown that DHPs do not affect Ca influx or Ca-dependent transmitter release in intact neurons (Ogura and Takahashi 1984; Shalaby et al. 1984; Boll and Lux 1985; Perney et al. 1986) or synaptosomes (Nachshen and Blaustein 1979; Ebstein and Daly 1982; Daniell et al. 1983; Rampe et al. 1984; Suszkiw et al. 1986). There is also evidence, however, that DHP-sensitive Ca channels are responsible for both Ca entry into and transmitter release from synaptosomes (Turner and Goldin 1985) and neurons or neuronal cell lines (Toll 1982; Takahashi and Ogura 1983; Freedman et al. 1984; Sasakawa et al. 1984.; Carboni et al. 1985; Enyeart et al. 1985; Middlemiss and Spedding

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Offprint requests to: S. G. Rane.

1985; Perney et al. 1986). Because of these conflicting reports, it is unclear whether or not DHP-sensitive Ca channels play an important role in controlling the release of neurotransmitters.

Primary sensory neurons from chick dorsal root ganglia (DRG) exhibit a slowly inactivating, voltage-dependent Ca current (termed L-type, Nowycky et al. 1985) which is similar to the DHP-sensitive cardiac Ca current (Lee and Tsien 1983). The release of the peptide transmitter substance P (SP) from DRG neurons appears to be controlled by L-type Ca channels in chick (Holz et al. 1987a, b) and rat (Perney et al. 1986). As an additional test of this hypothesis we studied the action of nifedipine on both Ca current measured under voltage clamp and the Ca-dependent release of SP from DRG neurons grown in culture. L-Type Ca current and the potassium-stimulated release of transmitter were both inhibited by nifedipine. However, as is the case for cardiac muscle (Bean 1984; Sanguinetti and Kass 1984), we found that the inhibitory action of nifedipine on the L-type Ca current was greatly enhanced when cells were held at relatively depolarized membrane potentials for prolonged durations. This voltage and time-dependent action of the drug may explain our observations that although nifedipine inhibited high-potassium stimulated SP release, it was ineffective in blocking SP release due to electrically-evoked action potentials.

Methods

Electrophysiology

For electrophysiological recording, DRG cells were plated onto 35 mm collagen-coated tissue culture dishes. To obtain cells, DRGs were first removed from 11-day chicken embryos, incubated at 37°C for 45 min in a Ca- and Mg-free saline (Puck's), and then mechanically dissociated by trituration through a fire-polished Pasteur pipette. Cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with heat-inactivated horse serum (10% vol/vol), chicken embryo extract (2.5%), 7 S nerve growth factor (1 µg/ml), penicillin (50 units/ml) and streptomycin (50 µg/ml). Proliferation of non-neuronal cells was halted with gamma-irradiation (5000 rads).

Whole cell voltage clamp currents were recorded with the patch electrode method of Hamill et al. (1981). The pipette solution contained (in mM): 150 CsCl, 10 HEPES, 5 MgATP, 5 BAPTA (5 bis-[0-aminophenoxy]ethane-N,N,-N',N'-tetraacetic acid; and the external solution contained (in mM): 133 NaCl, 1 CaCl₂, 0.8 MgCl₂, 10 tetraethyl-ammonium chloride, 25 HEPES, 12.5 NaOH, 5 glucose, 0.3 µM TTX. After the gigaseal was formed and whole cell access was established, Ca currents (evoked by voltage commands to 0 mV) were allowed to stabilize over 5–10 min before an experiment was begun. To minimize Ca current washout, the frequency of depolarizing commands was always 0.1 Hz or less. Currents, filtered at 10 kHz, and voltage commands were recorded and stored on FM tape. These records were later digitized at 20 kHz and analyzed with the aid of an LSI 11/23 microcomputer. Cells were used 7–22 days post-plating and experiments were performed at room temperature (21–25°C). A total of 27 cells were used for these experiments.

Action potentials from DRG neurons were recorded under current clamp with 2 M KCl-filled microelectrodes, and an amplifier equipped with an active bridge circuit for passing depolarizing current through the recording electrode. Action potential durations were measured directly from the oscilloscope traces as the duration at 1/2 peak spike amplitude. The bath solution for these recordings contained (in mM): 132 NaCl, 2.5 KCl, 2 CaCl₂, 1 BaCl₂, 0.8 MgCl₂ and 25 HEPES.

A stock solution of 10 mM nifedipine (Sigma, St. Louis, MO, USA) in 95% ethanol was prepared daily and the appropriate concentrations of nifedipine were prepared from this stock.

Nifedipine was pressure-applied from a blunt-tipped (3–5 μm), fire-polished pipette positioned about 20 μm from the cell (Choi and Fischbach 1981). Ethanol was added to the bath solution to give a final concentration equal to that in the puffer; however, application of as much as 0.05% ethanol alone had no effect on either DRG cell Ca current or the action potential.

Substance P release

For SP release experiments, DRG neurons were plated on 60 mm collagen-coated tissue culture dishes at a density of $1.5\text{--}2.0 \times 10^5$ neurons per dish. By 12–16 days post-plating the cultures contained 10–15 ng of SP-like immunoreactivity (SPLI) as measured by radio-immunoassay (RIA) of DRG cell extracts. SP antibody was provided by Dr. R. M. Kream, Dept. of Anesthesiology, Tufts New England Medical Center (Kream et al. 1985). High pressure liquid chromatography of DRG cell extracts demonstrated that the cellular content of SPLI is authentic SP. Therefore, SPLI will be referred to as SP. To determine the cellular content of SP, cultures were extracted in 6 N guanidine HCl and the extract was subjected to reverse-phase chromatography on Baker 10 SPE Octadecyl (C_{18}) columns. SP was eluted in 1 ml 50% acetonitrile containing 0.1% trifluoroacetic acid, and aliquots (20–50 μl) were lyophilized, resuspended in buffer and assayed for SP.

SP was released into the bathing solution in a Ca-dependent manner when cultures were depolarized by either 60 mM KCl or electrical field stimulation (Mudge et al. 1979; Holz et al. 1987a). For electrical field stimulation, cultures were bathed in HEPES-buffered saline (HBS, pH 7.4) containing (in mM): 132 NaCl, 2.5 KCl, 2 CaCl_2 , 1 BaCl_2 , 0.8 MgCl_2 , 25 HEPES and 0.04% heat-inactivated bovine serum albumin (BSA). Cultures were stimulated at room temperature using bipolar platinum/steel electrodes (square wave DC pulses, 3 ms duration, 110 V, 1 Hz for 5 min). Intracellular microelectrode recordings demonstrated that this stimulus intensity was suprathreshold to evoke action potentials in all neurons. For stimulation by elevated KCl solutions, cultures were bathed for 5 min in HBS containing (in mM): 74 NaCl, 60 KCl, 2 CaCl_2 , 1 BaCl_2 , 0.8 MgCl_2 , 25 HEPES and 0.04% BSA. This solution depolarized DRG neurons to ca. -25 mV. Nifedipine was prepared as a stock 5 mM solution in 50% ethanol that was diluted in HBS to obtain a 5 μM test solution. Control (vehicle-treated) solutions were prepared as HBS containing 0.05% ethanol.

Stimulation-induced release of SP was measured by direct radioimmunoassay (RIA) of the bathing solution. Cultures were stimulated while bathed in 1.7 ml HBS, and from this, 400 μl aliquots were assayed by RIA using a highly specific SP antibody and ^{125}I -labeled Bolton Hunter-conjugated SP tracer (Kream et al. 1985). RIA sensitivity was adjusted by varying the antibody dilution factor (typically 1:200,000). In most experiments the IC_{50} value for displacement of immunoreactive tracer by unlabeled SP was 250 pg per assay and the lower limit of detection was 10 pg/assay. Standard curves were generated by assaying known quantities of synthetic SP (Sigma) serially diluted in the same vehicle-treated HBS test solution as was used for the release experiments. Nifedipine (5 μM) did not interfere with binding of the tracer to the antibody (i.e. standard curves generated in control and nifedipine-treated HBS overlapped). The intra-assay coefficient of variation was $<10\%$.

Results

Electrophysiology

Effects of nifedipine on calcium current—We investigated the effects of the Ca channel antagonist nifedipine on voltage-dependent Ca currents recorded from chick DRG neurons in culture. When neurons were held at potentials near, or more negative than, rest (ca. -60 mV), step depolarizations to 0 mV evoked Ca currents of 2–5 nA. Application of 100 nM nifedipine caused only small reductions (ca. 10%) in these currents. In contrast, voltage clamp steps to 0

mV from a holding potential of -30 mV produced currents which were reduced an average of 40% by 100 nM nifedipine (Fig. 1). Higher concentrations of nifedipine (1 – 5 μ M) were only slightly more potent than 100 nM, but their effects were not readily reversible. Because partial reversibility could be observed following application of 100 nM nifedipine, the lower concentration was used for all the voltage clamp experiments described here.

The effect of nifedipine appears to be selective for high-threshold, long-lasting Ca currents (L-type). When Ca currents are recorded following depolarizations from holding potentials more negative than -60 mV, the resultant current displays an early transient peak and a time-dependent sag. The peak and the sag are both due to two rapidly inactivating Ca currents, 'T' and 'N', which are not available for activation at holding potentials more positive than about -60 mV (Carbone and Lux 1984; Nowycky et al. 1985). These two currents, first described in chick sensory neurons, appear as a small inactivating component superimposed on the much larger, non-inactivating L-type Ca current. For those cells held at -80 or -90 mV (Figs. 1 and 2, 9 cells in all) there was no apparent effect of nifedipine on the rapidly inactivating component of the whole cell current. Thus, in the presence of the drug there was a slight diminution of current at all times during the voltage step, but there was no change in the sag which reflects the inactivation of T and N-type Ca currents. Although we are conducting additional experiments on the fast-inactivating currents, our conclusion thus far is that nifedipine does not significantly reduce either T or N-type Ca currents in DRG cells. This result is consistent with the report that BAY K 8644, an agonist DHP, had no effect on T or N single channel Ca currents in DRG neurons (Nowycky et al. 1985).

Nifedipine does not affect the calcium-dependent action potential—The relative DHP-insensitivity of Ca channels observed for DRG cells held at normal resting potentials can also be observed under current clamp. DRG cells respond to depolarizing current pulses with action potentials which exhibit a prominent Ca-dependent plateau phase. The duration of this plateau can be decreased by agents which inhibit the voltage-dependent, L-type Ca current (Dunlap and Fischbach 1981). There was no significant effect of nifedipine (1 – 10 μ M) on action potential duration in the 18 cells tested. The average resting potential for these cells was -56 ± 1.3 mV and the average duration of the action potentials was 17 ± 2.1 ms. In light of the voltage-dependent action of nifedipine reported above, its ineffectiveness in reducing action potential duration is not surprising. As is evident from Fig. 2A, nifedipine caused minimal inhibition of Ca current when cells were depolarized from membrane potentials close to the normal resting potential. Even with depolarization from more positive membrane potentials, the onset of nifedipine-induced Ca current inhibition was slow relative to the time that a cell remains depolarized during the action potential (see below, Fig. 3A). Bay K 8644 (1 – 10 μ M), the agonist DHP which increases DRG cell L-type Ca current (Nowycky et al. 1985) and which is also effective at negative potentials for cardiac Ca current (Sanguinetti et al. 1986), increased DRG neuron action potential duration by ca. 350% (10 cells) (Holz et al. 1987a).

Voltage and time-dependent actions of nifedipine—The voltage-dependence of the nifedipine-induced inhibition of L-type Ca current was investigated using a standard steady-state inactivation protocol. Ca currents were measured during a 40 ms test pulse to 0 mV which was preceded by a 20 s prepulse command to potentials ranging from -80 to -20 mV. The duration of the prepulse was set at 20 s so as to produce nearly complete inactivation at -20 mV (see Fig. 3A). Test current amplitudes were plotted relative to the Ca current measured following a prepulse to -80 mV (defined as maximal current). Figure 2 A illustrates that peak Ca current measured during the test pulse decreases with increasing prepulse amplitude, with virtually complete inactivation following prepulses to -20 mV. This voltage step protocol was then repeated in the continuous presence of 100 nM nifedipine. For holding potentials between -50 and -20 mV, nifedipine caused about a 12 mV hyperpolarizing shift in the steady-state inactivation curve. This result is similar to the 15 mV hyperpolarizing shift observed by

Sanguinetti and Kass (1984) for the effect of 200 nM nisoldipine on Ca current in isolated calf Purkinje fibers. We conclude that, for a given membrane voltage, nifedipine enhances the inactivation of D R G neuron, L-type Ca channels, and that this effect is greatly accentuated as the membrane voltage is made more positive between approximately -65 and -20 mV.

The effect of nifedipine also exhibits a time-dependence (Fig. 3). For this experiment, cells were held at -80 mV and variable duration prepulses to -30 mV were applied, followed by 40 ms test depolarizations to 0 mV. Ca current was measured during the test pulse and plotted against pre-pulse duration. The entire voltage protocol was then repeated in the presence of 100 nM nifedipine. Test pulse current amplitudes decreased with increasingly longer duration prepulses, an effect which was enhanced by nifedipine. The relationship between current amplitude and prepulse duration is presumably a function of the rate at which Ca channels inactivate at -30 mV. Although it is not possible to determine, from this experiment, the precise mechanism by which Ca channels inactivate, it is clear that nifedipine either facilitates or stabilizes the inactivation process, and that this effect becomes more apparent the longer a cell is depolarized.

Substance P release

To determine whether the electrically-evoked release of SP could be modulated by nifedipine, 10 cultures from 2 platings were divided into 2 mixed sets of 5 vehicle-treated control cultures and 5 cultures treated with 5 μ M nifedipine. The amount of SP released into the bathing solution was calculated as a percentage of the total cellular content of SP per dish (total cellular content was calculated as the amount released plus the amount remaining in the cells).

As illustrated in Fig. 4A, prior to electrical stimulation the baseline levels of SP in the bathing solution were similar for control and nifedipine-treated cultures (0.4% and 0.35 % of the cellular content, respectively). Following electrical stimulation, the amount of SP released in control cultures was increased by approximately 24-fold over baseline, equivalent to the release of $9.60 \pm 0.6\%$ ($\pm$ SEM) of the total cellular content of SP/dish (in the experiments illustrated in Fig. 4A and B the average total cellular content was 13.8 ± 0.3 ng SP/dish, $n = 20$ cultures). When the second set of 5 cultures was electrically stimulated in the presence of 5 μ M nifedipine, $8.15 \pm 0.90\%$ of the total cellular content of SP/dish was released. This value was not significantly different from that observed for the control cultures ($p > 0.10$; paired Student's *t*-test), and it is consistent with our observation that nifedipine had no effect on the Ca-dependent action potential and L-type Ca currents evoked from negative holding potentials.

Since the action of nifedipine on Ca current is both voltage and time-dependent, we tested whether an inhibitory action of nifedipine on SP release might be observed under conditions of prolonged depolarization. For these experiments DRG cell cultures were depolarized with 60 mM KCl in the presence or absence of nifedipine and SP release was measured. Ten sibling cultures, from the same 2 platings used for the experiments illustrated in Fig. 4A, were divided into a set of 5 control cultures and a set of 5 cultures treated with nifedipine. Prior to depolarization, baseline levels of SP were determined during exposure to HBS containing 2.5 mM KCl. As illustrated in Fig. 4B, baseline levels of SP were similar for control and nifedipine-treated cultures (0.42% and 0.44% of the total cellular content, respectively). Following depolarization with 60 mM KCl for 5 min, the amount of SP released in control cultures was increased by approximately 9-fold over baseline, equivalent to $3.85 \pm 0.46\%$ of the total cellular content of SP/dish. In contrast, when the second set of 5 cultures treated with 5 μ M nifedipine was depolarized with 60 mM KCl, the amount of SP released was significantly reduced to 40% of control ($p < 0.001$), equivalent to the release of only $1.82 \pm 0.14\%$ of the total cellular content of SP/dish. The inhibitory action of nifedipine on substance P release was therefore revealed only under conditions of prolonged depolarization, consistent with the voltage and time-dependent inhibitory actions of nifedipine on Ca current as observed under voltage clamp.

Discussion

The results reported in this paper indicate that current flow through voltage-dependent L-type Ca channels in DRG neurons is inhibited by the antagonist DHP nifedipine. Furthermore, the action of this Ca channel antagonist was found to be voltage-dependent. This property of DHP inhibition of Ca current has been previously described for cardiac Ca channels (Bean 1984; Sanguinetti and Kass 1984). Thus, when cardiac cells are held at relatively depolarized membrane potentials (-20 to -45 mV), Ca current inhibition is observed with DHPs in the $1 - 100$ nM range. At more negative potentials (-70 to -80 mV), however, inhibition of Ca current is achieved only with 1000-fold higher DHP concentrations (Bean 1984). Our description of the voltage-dependence of DHP inhibition of DRG neuron Ca current may explain the lack of inhibitory effects of DHPs observed when Ca current or Ca-dependent action potentials are evoked following depolarizations from relatively negative holding potentials (Ogura and Takahashi 1984; Boll and Lux 1985).

We have further demonstrated that the release of SP from DRG neurons can be inhibited by nifedipine, provided that release is evoked under conditions of prolonged depolarization (i.e., high potassium stimulation). Since significant DHP inhibition of DRG cell Ca current occurs only with prolonged depolarizations (greater than 1 s), it is clear that sustained high-potassium depolarizations of several minutes are permissive for DHP inhibition of both Ca current and subsequent transmitter release. Conversely, the relative DHP-insensitivity of electrically-evoked SP release is consistent with the absence of DHP inhibition of L-type Ca channels in cells stimulated from their normal resting potentials. Thus, both the relatively negative resting potential (-60 mV) and the short duration (15 – 20 ms) Ca action potential of DRG neurons combine to minimize DHP-induced inhibition.

Several previous studies on neurons or neuronal cell lines have demonstrated DHP inhibition of Ca influx and transmitter release in response to potassium depolarization (Toll 1982; Takahashi and Ogura 1983; Freedman et al. 1984; Sasakawa et al. 1984; Carboni et al. 1985; Enyeart et al. 1985; Perney et al. 1986). In contrast to these effects on whole cell preparations, transmitter release from synaptosomes has been reported to be DHP-resistant (Nachshen and Blaustein 1979; Ebstein and Daly 1982; Daniell et al. 1983; Rampe et al. 1984; Suszkiw et al. 1986) although there is a report to the contrary (Turner and Goldin 1985). Due to the wide variety of experimental methods and protocols employed with these different preparations, it is difficult to directly compare the electrophysiological actions of the DHPs on neuronal Ca channels with the apparent DHP-insensitivity of the synaptosomal release process. It is possible that secretion from synaptosomal preparations is controlled by a DHP-resistant (non-L-type) Ca channel. However, the alternative possibility, that experimental conditions (e.g., extent and time-course of the depolarization or selective loss of DHP-sensitive Ca channels during tissue preparation) occlude DHP-sensitive Ca influxes, must be considered, particularly in light of a recent report of DHP inhibition of ^{45}Ca uptake and ^3H -norepinephrine release from synaptosomes (Turner and Goldin 1985). The results reported here indicate that only under certain conditions are DRG neuron L-type Ca channels DHP sensitive and, as such, suggest the limited usefulness of the DHPs as tools to assay the involvement of L-type Ca channels in other preparations not amenable to electrophysiological recording.

We have concentrated on the role of the slowly inactivating L-type Ca channel in transmitter release from chick DRG neurons. There are also two transient Ca currents present in these cells (Carbone and Lux 1984; Nowycky et al. 1985), although it is not known what role, if any, they play in neurosecretion. Our consistent finding is that agents which inhibit L-type current in DRG neurons also inhibit the release of SP. This is true not only for the DHP inhibition of K-evoked SP release reported here, but also for the GABA and norepinephrine-induced inhibition of electrically evoked SP release (Holz et al. 1987b). These two transmitters inhibit L-type Ca

channels and decrease the duration of the DRG cell action potential. Neither the transmitters nor the antagonist DHPs produce a complete inhibition of SP release, consistent with their partial inhibition of L-type Ca current. The DHP insensitivity of electrically-evoked SP release, reported here, likely results not from a lack of L-type Ca channel involvement in neurosecretion but rather from the conditional inhibitory action of the antagonist DHPs on L-type channels in DRG neurons.

Taken together, our results suggest that L-type Ca channels play a significant role in regulating SP release from DRG neurons. They do not rule out a role for transient Ca channels in neurosecretion, but they do suggest that due to the conditional block of L-current by the antagonist DHPs, DHP-insensitivity of the release process is not, by itself, sufficient evidence to rule out the involvement of L-type Ca channels.

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Fig. 1.

Nifedipine inhibition of neuronal calcium current is voltage-dependent. Amplitudes of dorsal root ganglion cell calcium currents were measured within the last 5 ms of a 40 ms voltage step to 0 mV from one of three holding potentials, -90, -60, or -30 mV. Voltage commands were given every 10 s and nifedipine was applied continuously for 30 s. Since complete reversibility was not achieved following drug application, each cell was tested at only a single holding potential. Representative control and nifedipine reduced current records are shown with the control records normalized to peak current measured in the cell held at -60 mV. For the three holding potentials, -90, -60, -30 mV (left to right), the actual control current amplitudes were -2.7, -3.4, and -1.5 nA respectively. The *calibration bar* represents 10 ms

Fig. 2.

A, B. Nifedipine affects steady-state inactivation of neuronal calcium current. **(A)** Nifedipine causes progressively greater attenuation of DRG cell calcium current as membrane holding potential is made more positive. This effect can be seen as a 12 mV hyperpolarizing shift in the steady-state inactivation curve. The durations of the prepotentials and the test pulse were 20 s and 40 ms respectively. Data points are averages ($\pm$ SEM) of normalized current amplitudes either in the presence ($\bullet$) or absence ($\blacksquare$) of 100 nM nifedipine. The maximal current amplitude was defined as the current produced from a holding potential of -80 mV either with or without nifedipine. The maximal current during nifedipine application was $86 \pm 4\%$ of the control current. Control results were obtained from 8 cells and nifedipine was applied to 4 of these cells. **(B)** Representative voltage clamp records show, for the same cell, the currents recorded at each of the 5 different holding potentials before (*left*) and after nifedipine application. Maximal current measured in the presence of nifedipine was normalized to control current amplitude to aid visual comparisons. The *calibration bars* represent 2 nA for control, 1.5 nA for drug, 10 ms for both. Linear washout of calcium current (less than 1%/min), which occurs under the whole cell dialysis conditions (Forscher and Oxford 1985 and unpublished data), makes some contribution to decreases in currents seen over the course of this and other long duration experiments

Fig. 3.

A, B. Nifedipine inhibition of neuronal calcium current is time-dependent. **(A)** Data points are averages ($\pm$ SEM) of normalized current amplitudes either with ($\bullet$) or without ($\blacksquare$) 100 nM nifedipine. The duration of the test pulse was 40 ms. The maximal current amplitude was defined as the current measured with a nominal prepulse duration (10 or 100 ms), with or without nifedipine. The maximal current during nifedipine application was $83 \pm 3\%$ of control. Results are averages from 4 cells. A one-tailed t -test was used to compare the average responses of nifedipine-treated and control cells (**, $p < 0.05$). **(B)** Superimposed representative currents following prepulses of 0.01, 1, 5, 10, 15, and 20 s before (*left*) and after application of 100 nM nifedipine. Scale bars indicate 3 nA for the control records and 2 nA for the nifedipine records, 10 ms for both

Fig. 4.

A, B. Nifedipine inhibits the 60 mM KCl-induced release of substance P from DRG neurons, but does not affect release induced by electrical field stimulation. **(A)** Nifedipine reduced the amount of substance P released in response to electrical field stimulation to 85% of the vehicle-treated control, but this inhibitory action was not statistically significant (n.s., $p > 0.10$; paired Student's t -test). **(B)** Nifedipine significantly reduced the amount of SP released in response to 60 mM KCl to 40% of control (**, $p < 0.001$). For both **(A)** and **(B)** the concentration of nifedipine was 5 μ M. Substance P release is expressed as a percentage of total cellular content prior to stimulation, and baseline release was subtracted from stimulated release to calculate percentage inhibition by the DHP