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# Nimodipine as a Potential Pharmacological Tool for Characterizing R-Type Calcium Currents

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Nimopidine, one of dihydropyridine derivatives, has been widely used to pharmacologically identify L-type Ca currents. In this study, it was tested if nimodipine is a selective blocker for L-type Ca currents in sensory neurons and heterologous system. In mouse dorsal root ganglion neurons (DRG), low concentrations of nimodipine ($<10\,\mu$M), mainly targeting L-type Ca currents, blocked high-voltage-activated calcium channel currents by ~38%. Interestingly, high concentrations of nimodipine ($>10\,\mu$M) further reduced the "residual" currents in DRG neurons from $\alpha_{1E}$ knock-out mice, after blocking L-, N- and P/Q-type Ca currents with 10 $\mu$M nimodipine, 1 $\mu$M $\omega$-conotoxin GVIA and 200 nM $\omega$-agatoxin IVA, indicating inhibitory effects of nimodipine on R-type Ca currents. Nimodipine ($>10\,\mu$M) also produced the inhibition of both low-voltage-activated calcium channel currents in DRG neurons and $\alpha_{1B}$ and $\alpha_{1E}$ subunit based Ca channel currents in heterologous system. These results suggest that higher nimodipine ($>10\,\mu$M) is not necessarily selective for L-type Ca currents. While care should be taken in using nimodipine for pharmacologically defining L-type Ca currents from native macroscopic Ca currents, nimodipine ($>10\,\mu$M) could be a useful pharmacological tool for characterizing R-type Ca currents when combined with toxins blocking other types of Ca channels.

Key Words: Dihydropyridines, Ca channels, Sensory neurons, R-type Ca currents

## INTRODUCTION

L-type Ca channels, one of the high-voltage-activated calcium channels (HVACC), are widely distributed in muscle, heart, neurosecretary cells and neurons. They are formed by different pore forming $\alpha_1$ subunits such as $\alpha_{1C}$, $\alpha_{1D}$, $\alpha_{1F}$ and $\alpha_{1S}$ in different type of cells (Catterall, 2000). In neurons, both $\alpha_{1C}$ and $\alpha_{1D}$ subunits contribute to L-type Ca currents (Chin et al, 1992).

HVACC currents in individual neurons, including sensory neurons, often result from N-, P/Q- and R-type Ca currents as well as L-type Ca currents (Acosta & Lopez, 1999), and which makes it difficult to quantify effects of a given drug on a single subtype of Ca channels. However, because different mechanisms are responsible for the modulation of certain neuronal VACC (Dolphin, 1998; Catterall, 2000; Bell et al, 2001), and different types of Ca channels play their own specific physiological roles in excitable cells (Catterall, 2000; Miller, 2001), the estimation of their relative contributions to HVACC currents could be very important, especially for developing therapeutic drugs targeting one of them. For this purpose, pharmacological tools, selectively targeting one of HVACCs, have been generally used.

Since L-type Ca channels are blocked by dihydropyridines (DHPs), phenylalkylamines and benzothiazepines with high affinity, these drugs have been used as pharmacological tools for isolating L-type Ca currents (Hockerman et al, 1997; Hering et al, 1998; Striessnig et al, 1998). Nimodipine is one of DHP antagonists, generally used to define L-type Ca cur-

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rents from HVACC currents. The portion of HVACC currents blocked by low concentration (nM to $\mu$M) of nimodipine was routinely classified as L-type Ca currents (McCarthy & TanPiengco, 1992). Recently, however, it has been demonstrated that neuronal $\alpha_{1D}$ subunit mediated L-type Ca currents are more resistant to DHP antagonists, and activated at the relatively hyperpolarized membrane potentials, compared to $\alpha_{1C}$ subunit mediated L-type Ca currents (Koschak et al, 2001; Xu & Lipscombe, 2001), suggesting potential use of DHPs for discriminating $\alpha_{1D}$ based L-type Ca currents from $\alpha_{1C}$ based L-type Ca currents. However, it has also been shown that certain DHPs block N-type Ca currents (Fujii et al, 1997; Furukawa et al, 1997), which suggests that DHPs may block other HVACC currents such as P/Q-, R-type, and low-voltage-activated calcium channel (LVACC) currents as well.

In the present study, it was investigated if nimodipine, especially at high concentrations ($>10\,\mu$M), produces inhibitory effects on the other types of HVACC currents and LVACC currents in mouse dorsal root ganglion (DRG) neurons and Ca channel expressing cell lines. Our data suggest that while care should be taken in using nimodipine for determining L-type Ca currents from native whole cell calcium currents, higher nimodipine ($>10\,\mu$M) could be a useful pharmacological tool for characterizing R-type Ca currents and a portion of R-type Ca currents may result from $\alpha_{1D}$ subunits.

## METHODS

### *Cell preparation*

Cultures of DRG neurons from neonatal mice were prepared with modification of the methods described previously (Thayer et al, 1988). Briefly, 2 to 5 day old mice or $\alpha_{1E}$ knock-out mice were decapitated, and the DRGs were dissected under aseptic conditions, after which they were treated sequentially with collagenase (Sigma, St. Louis. U.S.A.), collagenase/dispase (Boehringer Mannheim, Germany) and trypsin (Life Technologies, U.S.A.) in HBSS (Hank's balanced salt solution) (Life Technologies) for 10 minutes at 37°C. The digestion was halted by addition of HBSS and subsequent centrifugation (5 min, 1,000 rpm). The ganglia were resuspended in Ham's medium mixture F12 (Life Technologies), supplemented with 10% FBS (fetal bovine serum) (Atlanta Biologicals, U.S.A.), $N_2$ supplement (Life Technologies), 50 ng/ml NGF (nerve growth factor) (Colaborative Biomedical Products, U.S.A.), and penicillin and streptomycin (100 $\mu$g/ml and 100 U/ml, respectively), and dissociated into single cells by trituration through a series of heat-polished Pasteur pipettes. Several modifications were made to the protocol to minimize contamination of the cultures by non-neuronal cells. In most experiments, the cell suspension was replated on uncoated tissue culture dishes for 2 hr at 37°C. Nonadherent neuronal cells were dislodged from the dishes by gentle pipetting. The cells were then plated on polyornithine (Sigma)-and laminin (Collaborative Biomedical Products)-coated coverslips (25 mm diameter), and incubated in Ham's medium mixture F12 with the additives listed above except that FBS was reduced to 0.5%. The medium was replaced every 2~3 days. Finally, 1~10 $\mu$M cytosine arabinoside (Sigma) was added to inhibit the proliferation of non-neuronal cells including ganglionic fibroblasts. Cultures were maintained at 37°C in a water saturated atmosphere with 5% $CO_2$ for up to 2 weeks.

Two different cell lines that stably expresses N-type/$\alpha_{1B}$ based Ca channels, G1A1 (McCool et al, 1996) and C2D7 cells (McNaughton et al, 1998), and E52 cells, stably expressing $\alpha_{1E}$ based Ca channels, were maintained with conventional methods. On the day of recording, the cells were replated on poly-l-lysine coated coverslips.

### *Electrophysiological recordings*

The tight seal whole-cell configuration of patch clamp technique (Hamill et al, 1981) was used to record Ba currents ($I_{Ba}$) from individual cells. The patch electrodes were made of soft, soda-lime capillary glass and had resistances of 2~5 M$\Omega$ with pipette solution before seal formation. Pipette solution was composed of (mM): CsCl 100, $MgCl_2$ 1, HEPES 10, BAPTA 10, Mg-ATP 3.6, Phosphocreatine 14, GTP 0.1, creatine phosphokinase 50 units/ml, adjusted pH to 7.4 with CsOH. Extracellular solution contained (mM): Tetraethylammonium chloride (TEACl) 151, HEPES 10, $BaCl_2$ 5, $MgCl_2$ 1, glucose 10, adjusted to pH 7.4 with TEAOH. The osmolarity of the extracellular solution and internal standard solution was adjusted to 310~320 mosm and 290~300 mosm with sucrose, respectively. The $I_{Ba}$ was evoked by a

test pulse to +10 mV from a holding potential (−80 mV). To examine current-voltage relationship and record HVACC $I_{Ba}$ and LVACC $I_{Ba}$ at the same time, ramp command potentials, −80 to +10 from a Vh, −80 mV, were employed. Whole-cell currents were recorded with Axopatch-1D amplifier (Axon Instruments, U.S.A.). Partial series resistance compensation was employed and currents were lowpass filtered at 2 kHz, and sampled at 10 kHz. The pClamp6 (Axon Instruments, U.S.A.) software was used during experiments and analysis. Statistics are given as mean± SEM.

*Drugs*

Nimodipine was purchased from Sigma (St. Louis, U.S.A.). Nimodipine was dissolved in dimethylsulfoxide (DMSO) at 10 mM to make stock solution, and kept in light-proof container at −20°C. The final concentration of DMSO was less than 0.1% (v/v), which did not affect $I_{Ba}$. Just before experimentation, nimodipine was diluted to their final concentration using the extracellular solution, and then applied near the cells by local perfusion system. ω-Conotoxin GVIA and ω-agatoxin-IVA (Alomone labs, Israel) were dissolved in distilled water to make stock solution and stored at −20°C. These toxins were applied directly to the bath by a puff pipette. The bath solution was continuously applied during the experiment at a flow rate of 1 ml/min.

## RESULTS

*Nimodipine, at high concentrations, blocked HVACC currents other than L-type Ca channel currents in DRG neurons*

Whole-cell recording of Ca currents was performed, in cultured neonatal mice DRG neurons ranged from 15~40 μm in diameter, under experimental conditions that suppress other voltage-dependent currents, such as $Na^+$ and $K^+$ currents. 5 mM $Ba^{2+}$ was used as the charge carrier ($I_{Ba}$).

Because L-type Ca currents have been defined pharmacologically by their sensitivity to low (nM to μM) concentrations of 1,4-dihydropyridine (DHP) antagonists (e.g. nifedipine and nimodipine) and agonists (e.g., S-(-)-BayK8644) (Hockerman et al, 1997; Hering et al, 1998; Striessnig et al, 1998), the presence of L-type Ca currents was determined by their application. Fig. 1 shows representative traces of HVACC $I_{Ba}$ evoked by a test pulse to +10 mV from a holding potential (Vh) of −80 mV, in which the expression of L-type calcium channels on DRG neurons was clearly demonstrated. Whereas the $I_{Ba}$ was enhanced by the application of 5 μM (-)-BayK8644, 10 μM nimodipine, mainly targeting $\alpha_{1C}$ mediated L-type HVACC currents, reduced $I_{Ba}$ by 38 ±5% (n=12) (Fig. 1A). These effects were reversible.

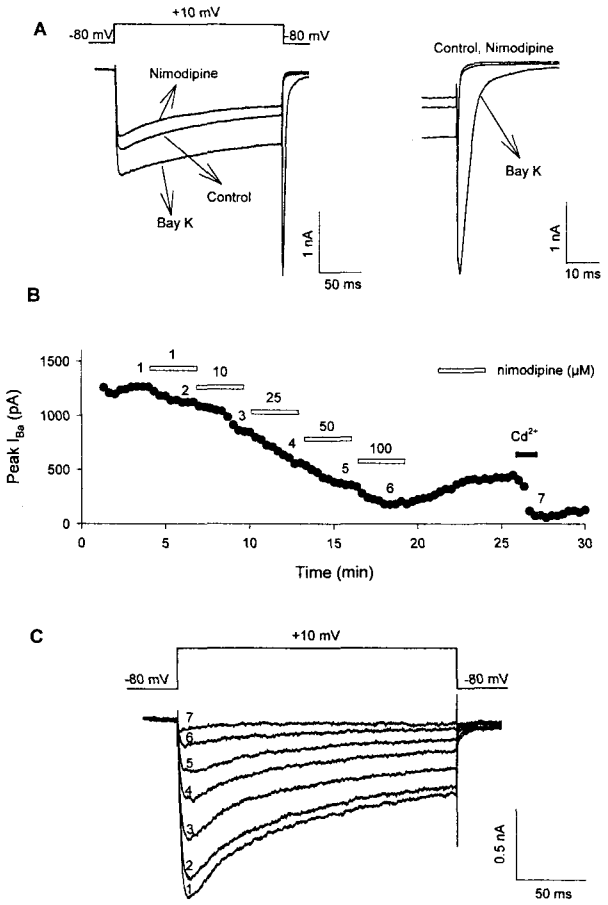


**Fig. 1.** Effects of nimodipine on HVACC $I_{Ba}$ in DRG neurons from neonatal mice. (A) The current traces show that while DHP antagonist, nimodipine (10 μM) inhibited L-type Ca current, DHP agonist (-)-Bay K 8644 (5 μM) enhanced L-type Ca current. (-)-Bay K8644 induced slow tail current, but nimodipine did not. Currents were evoked by a 200 msec step from −80 mV to +10 mV. (B) Time course of peak $I_{Ba}$ inhibition by the serial application of nimodipine on DRG neurons. Higher nimodipine further blocked HVACC $I_{Ba}$. $Cd^{2+}$ (25 μM) completely blocked the $I_{Ba}$. (C) Superimposed current traces at the points indicated in B.

(-)-BayK8644 showed marked effect on deactivating tail currents also, inducing slow component of tail currents (Fig. 1A). This phenomenon results from prolonged openings of L-type Ca channels (Nowycky et al, 1985) and which is characteristic of L-type channels in many cells (Rusin & Moises, 1995). Higher concentration of nimodipine was then tested whether it has more effects on the HVACC $I_{Ba}$. When DRG neurons were exposed to higher than 10 $\mu$M nimodipine, the remaining $I_{Ba}$ was further blocked (Fig. 1C), indicating inhibitory effects of nimodipine on HVACCs other than $\alpha_{1C}$ mediated L-type Ca channels.

*Nimodipine blocked R-type Ca currents in DRG neurons*

The $\alpha_{1E}$ Ca channel subunits, which had been hypothesized to mainly contribute to R-type Ca currents, have been demonstrated to contribute only minor portion of R-type Ca currents in mouse DRG neurons (Wilson et al, 2000). However, pharmacological tools for characterizing Ca channel subunits other than $\alpha_{1E}$ subunits that result in the majority of R-type Ca currents are yet to be developed. Because R-type Ca currents contributed to the HVACC currents that were blocked by higher nimodipine (>10 $\mu$M) in DRG neurons (Fig. 1C), it was examined if nimodipine has selective effect on R-type Ca current in $\alpha_{1E}$ knock-out mice. Using these knock-out mice, the contribution of $\alpha_{1E}$ subunits to R-type Ca currents were excluded. After blocking N-, P/Q- and L-type HVACC currents with saturating concentration of drugs, 1 $\mu$M $\omega$-conotoxin GVIA, 200 nM $\omega$-agatoxin-IVA and 10 $\mu$M nimodipine, respectively, the residual currents in DRG neurons constituted R-type Ca currents resulting from $\alpha_1$ subunits other than $\alpha_{1E}$ subunits. When tested, higher concentration of nimodipine (>10 $\mu$M) inhibited R-type Ca currents in a dose-dependent manner (Fig. 2A, 2B, 2C). These electrophysiological data indicated that nimodipine could be a useful pharmacological tool for characterizing $\alpha_1$ subunits contributing to R-type Ca currents.

*Nimodipine blocked low-voltage-activated calcium channel (LVACC) currents in DRG neurons*

The fact that higher nimodipine has inhibitory effects on HVACC currents including R-type Ca currents (Fig. 1C) leads us to examine if it has effects on LVACC currents as well. In a subset of DRG neurons, LVACC $I_{Ba}$ was activated together with HVACC $I_{Ba}$ when evoked with ramp command potential, −80 to +10 mV, from a holding potential (Vh), −80 mV. Fig. 3 shows a representative response of DRG neurons to serial application of nimodipine over a range of concentrations. While low concentration of nimodipine (<10 $\mu$M) did not have

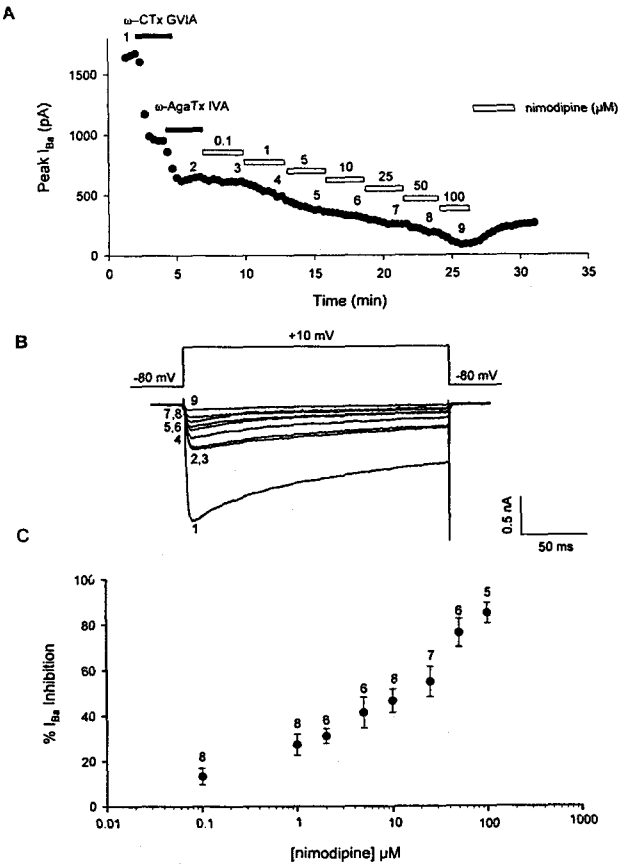


**Fig. 2.** Effects of nimodipine on HVACC $I_{Ba}$ in DRG neurons from $\alpha_{1E}$ knock-out mice. (A) Time course of peak $I_{Ba}$ inhibition produced by toxins and nimodipine. After blocking N- and P/Q-type Ca currents with saturating dosage of $\omega$-conotoxin GVIA and $\omega$-agatoxin IVA, the residual currents were blocked by nimodipine over a wide range of concentrations. (B) The current traces show the superimposed $I_{Ba}$ at the points indicated in A. Currents were evoked by a 200 msec step from −80 mV to +10 mV. (C) Dose-response relationship for the $I_{Ba}$ inhibition produced by nimodipine, after blocking N- and P/Q-type Ca currents with 1 $\mu$M $\omega$-conotoxin GVIA and 200 nM $\omega$-agatoxin IVA, indicating inhibitory effects of nimodipine on R-type Ca current in DRG neurons.

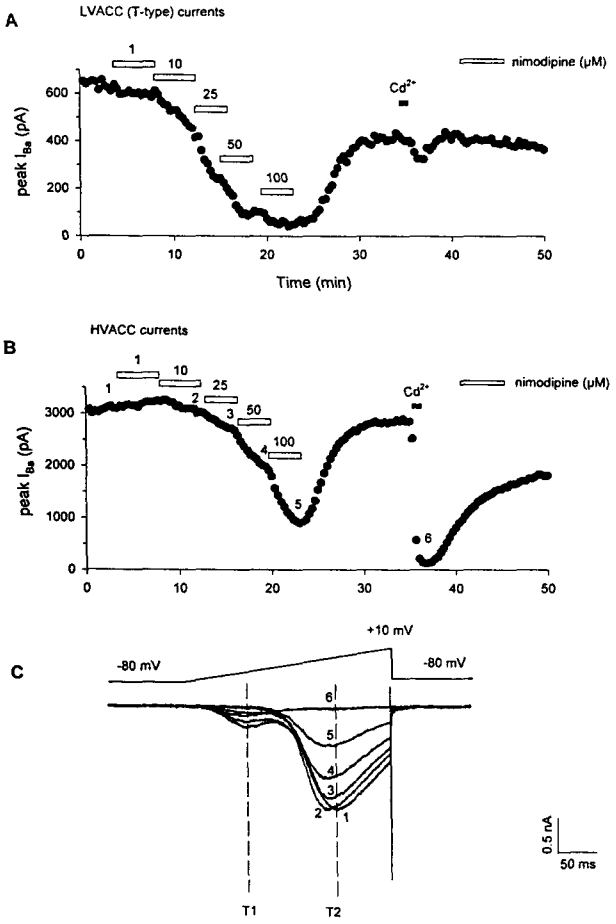


**Fig. 3.** Effects of nimodipine on LVACC $I_{Ba}$ in DRG neurons from $\alpha_{1E}$ knock-out mice. (A) Time course of LVACC $I_{Ba}$ inhibition by nimodipine over a range of concentrations at the time point (T1) indicated in C. LVACC $I_{Ba}$ was blocked by higher concentrations ($>10$ $\mu$M) of nimodipine. Notice that LVACC $I_{Ba}$ was only partially blocked by $Cd^{2+}$ (25 $\mu$M). (B) Time course of HVACC $I_{Ba}$ inhibition by nimodipine over a range of concentrations at the time point (T2) indicated in C. Notice that HVACC $I_{Ba}$ was completely blocked by $Cd^{2+}$ (25 $\mu$M). (C) Illustration of the ramp protocol employed in this experiments and superimposed current traces at the points indicated in B. The traces show both LVACC $I_{Ba}$ and HVACC $I_{Ba}$.

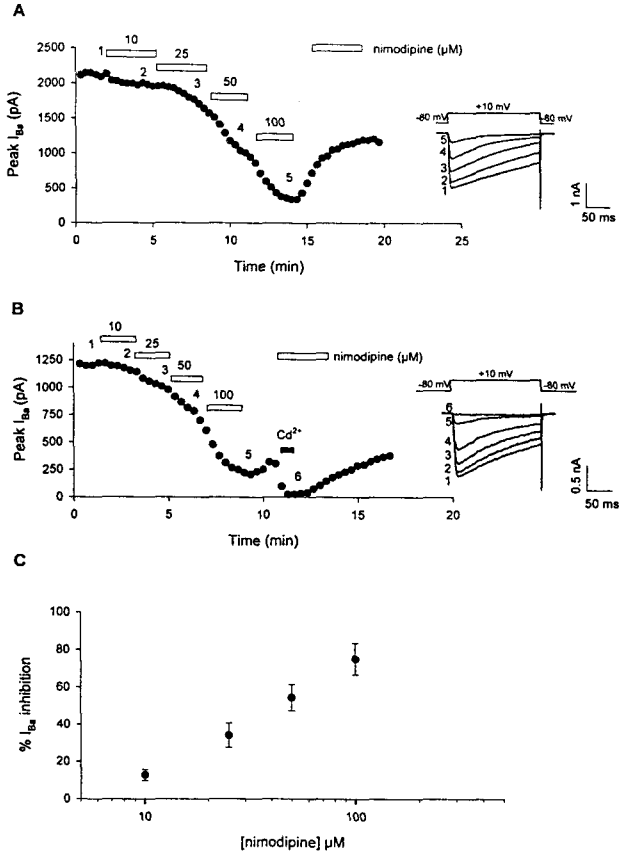


**Fig. 4.** Effects of nimodipine on N-type/ $\alpha_{1B}$ based $Ca^{2+}$ channels. (A) Time course of peak $I_{Ba}$ inhibition by the serial application of nimodipine in C2D7 cells. N-type Ca channel currents were blocked by nimodipine at higher than 10 $\mu$M. *Inset*, The current traces show the superimposed $I_{Ba}$ at the points indicated in the plot. (B) Time course of peak $I_{Ba}$ inhibition by the serial application of nimodipine in G1A1 cells. N-type Ca channels stably expressed in these cells were also blocked by higher than 10 $\mu$M nimodipine. *Inset*, The current traces show the superimposed $I_{Ba}$ at the points indicated in the plot. Currents were evoked by a 200 msec step from $-80$ mV to $+10$ mV. $Cd^{2+}$ (25 $\mu$M) completely blocked N-type Ca channels. (C) Dose-response relationship for N-type Ca channel currents inhibition produced by nimodipine.

clear effects on $I_{Ba}$, higher concentration of nimodipine produced the inhibition of both LVACC $I_{Ba}$ and HVACC $I_{Ba}$ in this cell. Consistent with other report (Hilaire et al, 1997), $Cd^{2+}$ (25 $\mu$M) partially inhibited LVACC currents, but HVACC currents were completely blocked by $Cd^{2+}$ (25 $\mu$M). Because $\omega$-conotoxin GVIA and $\omega$-agatoxin-IVA were not added in the bath solution, the portions of HVACC currents blocked by nimodipine over a wide range of concentrations included $\omega$-conotoxin GVIA/ $\omega$-agatoxin-IVA sensitive currents, N- and P/Q-type currents.

### *Nimodipine blocked $\alpha_{1B}$ based $I_{Ba}$*

From the results of DRG neurons described above, higher nimodipine ($>10$ $\mu$M) seems to have non-

specific actions on HVACC $I_{Ba}$ other than L-type Ca currents. Therefore, two cells lines, C2D7 cells and G1A1 cells which stably express N-type/$\alpha_{1B}$ based $Ca^{2+}$ channels (McCool et al, 1996; McNaughton et al, 1998), were examined to see if nimodipine also blocked N-type Ca channel currents. Fig. 4 depicts representative responses of both cell lines to a serial application of nimodipine. Nimodipine clearly inhibited N-type Ca channel currents in a dose-dependent manner (Fig. 4C). At the concentration lower than 10 $\mu$M, which was known to target mainly L-type Ca channels, nimodipine did not inhibit the $I_{Ba}$. In contrast, 10 $\mu$M nimodipine decreased the $I_{Ba}$ to 13±3% of control, and 100 $\mu$M nimodipine blocked the $I_{Ba}$ by 75±8% of control (n=6).

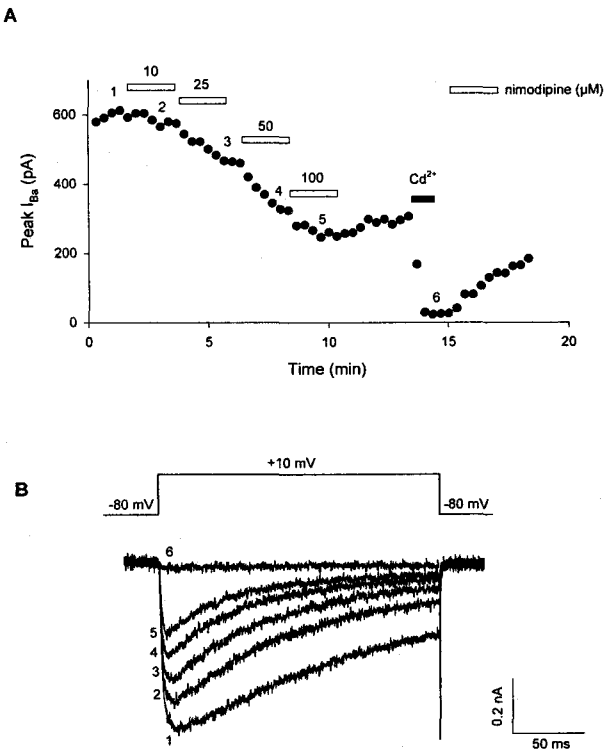


**Fig. 5.** Effects of nimodipine on $\alpha_{1E}$ based $Ca^{2+}$ channels in E52 cells. (A) Time course of peak $I_{Ba}$ inhibition produced by the serial application of nimodipine. Data show that nimodipine at higher than 10 $\mu$M inhibited $\alpha_{1E}$ based $Ca^{2+}$ channels in this cell. $Cd^{2+}$ (25 $\mu$M) completely blocked the $I_{Ba}$. (B) The current traces show the superimposed $I_{Ba}$ at the points indicated in A. Currents were evoked by a 200 msec step from −80 mV to +10 mV.

*Nimodipine blocked $\alpha_{1E}$ based $I_{Ba}$*

The effects of nimodipine on $\alpha_{1E}$ based Ca channels were also examined in E52 cells-a cell line stably expressing $\alpha_{1E}$ based Ca channels. Because it turned out that $\alpha_{1E}$ Ca channel subunits contribute only minor portion of the R-type Ca currents, $\alpha_{1E}$ mediated Ca currents were not mentioned as R-type Ca currents in this paper.

As it does in $\alpha_{1B}$ based Ca channel expressing cell lines, higher concentration of nimodipine (>10 $\mu$M) blocked $\alpha_{1E}$ based $I_{Ba}$ in these cells (Fig. 5), demonstrating that nimodipine (>10 $\mu$M) had potential non-selective actions on HVACC currents.

## DISCUSSION

Several different types of Ca channels coexist within the same neurons, contributing to the native macroscopic HVACC currents, and each Ca channel $\alpha_1$ subunit participates in its own physiological roles (Catterall, 2000). In sensory neurons, certain types of Ca channels have important roles in the nociceptive signal transduction. For example, N-type Ca channels are involved in glutaminergic transmission between DRG neurons and spinal dorsal horn (Gruner & Silva, 1994), and L-type Ca channels are important for the release of substance P from DRG neurons (Holz et al, 1989). Therefore, it is important to know the relative contribution of each subtype of Ca channels to native currents and their differential distribution in DRG neurons, especially for developing potential analgesic drugs targeting one of them.

It has been electrophysiologically demonstrated that at least five different types of voltage-activated Ca channels-L-, N-, P/Q-, R- and T-type channels, exist in rodent DRG neurons (Scroggs & Fox, 1992; Acosta & Lopez, 1999). Yusaf et al (2001) have shown the differential expression of certain pore forming $\alpha_1$ subunits of each Ca channels- $\alpha_{1A}$ (P/Q-type), $\alpha_{1B}$ (N-type), $\alpha_{1C}$, $\alpha_{1D}$, $\alpha_{1S}$ (L-type), $\alpha_{1E}$ (R-type) and $\alpha_{1I}$ (T-type) in rat DRG neurons. Consistent with this report, $\alpha_{1D}$ subunits have been demonstrated to contribute to L-type Ca currents in rodent DRG neurons (Wyatt et al, 1997). Recent immunohistochemical study also demonstrated that whereas $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1E}$ subunits were found to heterogeneously label DRG neurons, the distributions of $\alpha_{1C}$ and $\alpha_{1D}$ were similar and relatively homo-

geneous throughout the DRG neurons (Murakami et al, 2001). These results indicate that both $\alpha_{1C}$ and $\alpha_{1D}$ expressed in the same cells may contribute to L-type Ca currents. However, although DHP derivatives such as nimodipine have been widely used to define L-type Ca currents in excitable cells (McCarthy & TanPiengco, 1992), it is difficult to isolate $\alpha_{1C}$ mediated currents from $\alpha_{1D}$ mediated currents, so is the relative contribution of $\alpha_{1C}$ or $\alpha_{1D}$ subunits to L-type Ca currents. Recently, it has been shown that neuronal L-type Ca currents resulting from $\alpha_{1D}$ subunits are more resistant to DHP antagonists and activated at the relatively hyperpolarized membrane potentials (Koschak et al, 2001; Xu & Lipscombe, 2001), compared to those resulting from $\alpha_{1C}$ subunits. Therefore, differential sensitivity of $\alpha_{1C}$ and $\alpha_{1D}$ subunits to DHPs could be used for this purpose.

Our electrophysiological data from DRG neurons suggest this may be the case (Fig. 1, 2). L-type Ca currents in DRG neurons (Fig. 1) were determined by their sensitivity to low concentration of nimodipine ($<10\,\mu$M) and assumed to result from mainly $\alpha_{1C}$ subunits. Given the fact that $10\,\mu$M nimodipine inhibited only 13% of N-type Ca currents in C2D7 cells and G1A1 cells (Fig. 4), nonspecific actions of nimodipine (up to $10\,\mu$M) on HVACC currents other than L-type Ca currents in DRG neurons seem to be ignorable. Interestingly, the "residual" currents (i.e. R-type Ca currents), after blocking L, N- and P/Q-type Ca currents with $10\,\mu$M nimodipine, $1\,\mu$M $\omega$-conotoxin GVIA and 200 nM $\omega$-agatoxin IVA in DRG neurons from $\alpha_{1E}$ knock- out mice, were further blocked by the application of higher concentrations of nimodipine (Fig. 2). Because the "residual" currents were more resistant to nimodipine, compared to classic L-type Ca currents, they may result from the expression of $\alpha_{1D}$ subunits in DRG neurons (Koschak et al, 2001; Xu & Lipscombe, 2001). In addition, because we have recently demonstrated that the majority of R-type Ca currents result from the expression of Ca channel $\alpha_1$ subunits other than $\alpha_{1E}$ subunits (Wilson et al, 2000), R-type Ca currents are more likely to be based on $\alpha_{1D}$ subunits than $\alpha_{1E}$ subunits in mouse DRG neurons. Nevertheless, this is somewhat too early to conclude yet. Because it was not practically feasible to determine a particular concentration of nimodipine that isolates $\alpha_{1C}$ mediated currents from $\alpha_{1D}$ mediated currents, the voltage shift for the activation of $\alpha_{1D}$ mediated L-type Ca currents, which has been demonstrated in other studies (Koschak et al, 2001; Xu & Lipscombe, 2001), was hardly observed in native currents from DRG neurons (data not shown). Indeed, it is likely that $\alpha_{1D}$ subunits also contribute, at least in part, to the L-type Ca currents blocked by $10\,\mu$M nimodipine. According to Xu and Lipscombe (2001), although $\alpha_{1D}$ based L-type Ca currents are ~20-fold less sensitive to nimodipine, they were also inhibited by $10\,\mu$M nimodipine in heterologous system. In addition, at higher concentrations ($>10\,\mu$M), nimodipine may have non-specific effects on other Ca channels as well.

In G1A1 cells and C2D7 cells, as well as E52 cells, the higher concentration of nimodipine, which was the same concentration used for characterizing R-type Ca currents from $\alpha_{1E}$ knock-out mice, also blocked both $\alpha_{1B}$ based Ca currents and $\alpha_{1E}$ based Ca currents, respectively (Fig. 4, 5). These results suggest that higher concentration of nimodipine ($>10\,\mu$M) is not necessarily selective for $\alpha_{1D}$ subunits, but also block $\alpha_1$ subunits other than $\alpha_{1D}$, as shown by other reports (Diochot et al, 1995; Fujii et al, 1997; Furukawa et al, 1997; Furukawa et al, 1999). In addition, because LVACC currents were also blocked by higher nimodipine (Fig. 3), it seems indisputable that nimodipine has non-selective inhibitory actions on Ca channels. Indeed, it has been demonstrated that DHPs block T-type Ca channels (Richard et al, 1991; Formenti et al, 1993), and even $K^+$ and $Na^+$ channels (Jones & Jacobs, 1990; Fagni et al, 1994). If it is true that nimodipine, at higher than $10\,\mu$M, has non-specific effects on ion channels including HVACCs, the "residual" currents blocked by nimodipine in DRG neurons from $\alpha_{1E}$ knock-out mice are not necessarily to be based on the $\alpha_{1D}$ subunits. They may reflect the expression of unidentified HVACC $\alpha_1$ subunits and/or other isoforms of the $\alpha_{1C}$ and $\alpha_{1D}$ subunits that are poorly sensitive to nimodipine.

In summary, $\alpha_{1D}$ based Ca currents may contribute to R-type Ca currents in mouse DRG neurons. However, because nimodipine at higher concentrations ($>10\,\mu$M) has potential non-specific actions on other Ca channels as well, further studies using other experimental paradigms such as $\alpha_{1D}$/ $\alpha_{1E}$ double knock-out mice are still required to confirm whether $\alpha_{1D}$ based Ca currents constitute the major portion of R-type Ca currents or not. Besides, our data suggest that care should be taken in using nimodipine as a tool for pharmacologically characterizing L-type Ca currents from native macroscopic calcium currents.

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## REFERENCES

Acosta CG, Lopez HS. delta opioid receptor modulation of several voltage-dependent Ca (2+) currents in rat sensory neurons. *J Neurosci* 19: 8337−8348, 1999

Bell DC, Butcher AJ, Berrow NS, Page KM, Brust PF, Nesterova A, Stauderman KA, Seabrook GR, Nurnberg B, Dolphin AC. Biophysical properties, pharmacology, and modulation of human, neuronal L-type (alpha(1D), Ca(V)1.3) voltage-dependent calcium currents. *J Neurophysiol* 85: 816−827, 2001

Catterall WA. Structure and regulation of voltage-gated Ca2+ channels. *Annu Rev Cell Dev Biol* 16: 521−555, 2000

Chin H, Smith MA, Kim HL, Kim H. Expression of dihydropyridine-sensitive brain calcium channels in the rat central nervous system. *FEBS Lett* 299: 69−74, 1992

Diochot S, Richard S, Baldy-Moulinier M, Nargeot J, Valmier J. Dihydropyridines, phenylalkylamines and benzothiazepines block N-, P/Q- and R-type calcium currents. *Pflugers Arch* 431: 10−19, 1995

Dolphin AC. Mechanisms of modulation of voltage-dependent calcium channels by G proteins. *J Physiol* 506: 3−11, 1998

Fagni L, Bossu JL, Bockaert J. Inhibitory effects of dihydropyridines on macroscopic K+ currents and on the large-conductance Ca(2+)-activated K+ channel in cultured cerebellar granule cells. *Pflugers Arch* 429: 176−182, 1994

Formenti A, Arrigoni E, Mancia M. Low-voltage activated calcium channels are differently affected by nimodipine. *Neuroreport* 5: 145−147, 1993

Fujii S, Kameyama K, Hosono M, Hayashi Y, Kitamura K. Effect of cilnidipine, a novel dihydropyridine Ca++-channel antagonist, on N-type Ca++ channel in rat dorsal root ganglion neurons. *J Pharmacol Exp Ther* 280: 1184−1191, 1997

Furukawa T, Nukada T, Suzuki K, Fujita Y, Mori Y, Nishimura M, Yamanaka M. Voltage and pH dependent block of cloned N-type Ca2+ channels by amlodipine. *Br J Pharmacol* 121: 1136−1140, 1997

Furukawa T, Yamakawa T, Midera T, Sagawa T, Mori Y, Nukada T. Selectivities of dihydropyridine derivatives in blocking Ca(2+) channel subtypes expressed in Xenopus oocytes. *J Pharmacol Exp Ther* 291: 464−473, 1999

Gruner W, Silva LR. Omega-conotoxin sensitivity and presynaptic inhibition of glutamatergic sensory neurotransmission in vitro. *J Neurosci* 14: 2800−2808, 1994

Hamill OP, Marty A, Neher E, Sakmann B, Sigworth FJ. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflugers Arch* 391: 85−100, 1981

Hering S, Berjukow S, Aczel S, Timin EN. Ca2+ channel block and inactivation: common molecular determinants. *Trends Pharmacol Sci* 19: 439−443, 1998

Hilaire C, Diochot S, Desmadryl G, Richard S, Valmier J. Toxin-resistant calcium currents in embryonic mouse sensory neurons. *Neuroscience* 80: 267−276, 1997

Hockerman GH, Peterson BZ, Johnson BD, Catterall WA. Molecular determinants of drug binding and action on L-type calcium channels. *Annu Rev Pharmacol Toxicol* 37: 361−396, 1997

Holz GGt, Kream RM, Spiegel A, Dunlap K. G proteins couple alpha-adrenergic and GABAb receptors to inhibition of peptide secretion from peripheral sensory neurons. *J Neurosci* 9: 657−666, 1989

Jones SW, Jacobs LS. Dihydropyridine actions on calcium currents of frog sympathetic neurons. *J Neurosci* 10: 2261−2267, 1990

Koschak A, Reimer D, Huber I, Grabner M, Glossmann H, Engel J, Striessnig J. alpha 1D (Cav1.3) subunits can form l-type Ca2+ channels activating at negative voltages. *J Biol Chem* 276: 22100−22106, 2001

McCarthy RT, TanPiengco PE. Multiple types of high-threshold calcium channels in rabbit sensory neurons: high-affinity block of neuronal L-type by nimodipine. *J Neurosci* 12: 2225−2234, 1992

McCool BA, Pin JP, Brust PF, Harpold MM, Lovinger DM. Functional coupling of rat group II metabotropic glutamate receptors to an omega-conotoxin GVIA-sensitive calcium channel in human embryonic kidney 293 cells. *Mol Pharmacol* 50: 912−922, 1996

McNaughton NC, Bleakman D, Randall AD. Electrophysiological characterisation of the human N-type Ca2+ channel II: activation and inactivation by physiological patterns of activity. *Neuropharmacology* 37: 67−81, 1998

Miller RJ. Rocking and rolling with Ca(2+) channels. *Trends Neurosci* 24: 445−449, 2001

Murakami M, Suzuki T, Nakagawasai O, Murakami H, Murakami S, Esashi A, Taniguchi R, Yanagisawa T, Tan-No K, Miyoshi I, Sasano H, Tadano T. Distribution of various calcium channel alpha(1) subunits in murine DRG neurons and antinociceptive effect of omega-conotoxin SVIB in mice. *Brain Res* 903: 231−236, 2001

Nowycky MC, Fox AP, Tsien RW. Long-opening mode of gating of neuronal calcium channels and its promotion by the dihydropyridine calcium agonist Bay K 8644. *Proc Natl Acad Sci USA* 82: 2178−2182, 1985

Richard S, Diochot S, Nargeot J, Baldy-Moulinier M, Valmier J. Inhibition of T-type calcium currents by dihydropyridines in mouse embryonic dorsal root ganglion neurons. *Neurosci Lett* 132: 229−234, 1991

Rusin KI, Moises HC. mu-Opioid receptor activation reduces multiple components of high-threshold calcium current in rat sensory neurons. *J Neurosci* 15: 4315−4327, 1995

Scroggs RS, Fox AP. Calcium current variation between acutely isolated adult rat dorsal root ganglion neurons of different size. *J Physiol* 445: 639−658, 1992

Striessnig J, Grabner M, Mitterdorfer J, Hering S, Sinnegger MJ, Glossmann H. Structural basis of drug binding to L Ca2+ channels. *Trends Pharmacol Sci* 19: 108−115, 1998

Thayer SA, Perney TM, Miller RJ. Regulation of calcium homeostasis in sensory neurons by bradykinin. *J Neurosci* 8: 4089−4097, 1988

Wilson SM, Toth PT, Oh SB, Gillard SE, Volsen S, Ren D, Philipson LH, Lee EC, Fletcher CF, Tessarollo L, Copeland NG, Jenkins NA, Miller RJ. The status of voltage-dependent calcium channels in alpha 1E knockout mice. *J Neurosci* 20: 8566−8571, 2000

Wyatt CN, Campbell V, Brodbeck J, Brice NL, Page KM, Berrow NS, Brickley K, Terracciano CM, Naqvi RU, MacLeod KT, Dolphin AC. Voltage-dependent binding and calcium channel current inhibition by an anti-alpha 1D subunit antibody in rat dorsal root ganglion neurones and guinea-pig myocytes. *J Physiol* 502: 307−319, 1997

Xu W, Lipscombe D. Neuronal Ca(V)1.3alpha(1) L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. *J Neurosci* 21: 5944−5951, 2001

Yusaf SP, Goodman J, Pinnock RD, Dixon AK, Lee K. Expression of voltage-gated calcium channel subunits in rat dorsal root ganglion neurons. *Neurosci Lett* 311: 137−141, 2001