The Calcium Channel Blocker Nitrendipine Blocks Sodium Channels in Neonatal Rat Cardiac Myocytes

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SUMMARY. The dihydropyridine calcium channel blocker, nitrendipine, was studied for its effects on the sodium current of single cultured ventricular cells from neonatal rats. The patch-clamp method of recording whole cell currents was used, and sodium currents were isolated by suppressing potassium and calcium currents. Potassium currents were blocked by replacing potassium with cesium in the internal and external solutions and by adding tetraethylammonium chloride and 4-aminopyridine in the external solution; calcium current was blocked by replacing calcium with cobalt in the external solution. At low frequencies (0.1 Hz), nitrendipine reduced sodium currents without any significant change in the current-voltage relation. The block was dose dependent, and assuming a single occupancy model with complete block, had a half-maximum value of \(3 \times 10^{-8} \text{M} \) at a holding potential of \(-80 \text{mV} \) where half the sodium channels are activatable. This value is within the range of the \(K_d\)'s that have been reported for low-affinity dihydropyridine-binding sites found in cardiac sarcolemmal vesicles. In the presence of nitrendipine, the inactivation curve was shifted to hyperpolarized potentials. The block was greater with pulse intervals shorter than 1000 msec, and repriming was prolonged in the presence of the drug. These effects are similar to those of local anesthetics of the tertiary amine class, such as lidocaine. The block was relieved by the dihydropyridine agonist Bay K8644. The results are interpreted as indicating that dihydropyridines react with sodium channels.

ELECTROPHYSIOLOGICAL studies have shown that \(Ca^{++}\) channel blockers affect other membrane currents as well. For example, the organic \(Ca^{++}\) channel blocker, D600, and the inorganic \(Ca^{++}\) channel blockers, \(Mn^{++}\) and \(La^{+++}\), reduce outward currents in Purkinje fibers (Kass and Tsien, 1975; Siegelbaum and Tsien, 1980). D600 and verapamil have recently been shown to block \(Na^{+}\) channels in neuronal as well as cardiac cells (Galper and Catterall, 1979; Frelin et al., 1982). The lack of specificity is not restricted to \(Ca^{++}\) channel blockers. Organic \(Na^{+}\) channel blockers such as the local anesthetic group of drugs have effects on \(K^{+}\) currents (Weld and Bigger, 1976). \(Ca^{++}\) channels in neuronal cells are also blocked by what were thought to be \(Na^{+}\) channel-specific drugs, such as veratridine, batrachotoxin, and other agents (Romey and Lazdunski, 1982).

Recently, a new group of \(Ca^{++}\) channel blockers, the 1,4-dihydropyridines (DHP), of which nifedipine and nitrendipine are examples, has been shown to block \(Ca^{++}\) currents \(I_{Ca}\) in some smooth muscle, with a very low half-maximal inhibition \(I_{Ca}\) value (Triggle and Swamy, 1983; Fleckenstein, 1983). The DHP also block inactivated \(Ca^{++}\) channels in the heart with high potency, as well (Sanguinetti and Kass, 1984; Brown et al., 1984a; Bean, 1984). Receptor-binding studies have shown that DHP bind to a very high affinity \(K_d\) of about 0.1 nM) site in plasma membranes of many excitable cells from tissues including heart, brain, and smooth muscle (for cardiac sarcolemmal vesicles, see Bellemann et al. (1981), Ehlert et al. (1982), Williams and Tremble (1982), DePover et al. (1982), Murphy and Snyder (1982); for cardiac cells, see Marsh et al. (1983)). In addition, DHP bind to a lower affinity site in these tissues \(K_d\) of about 100 nM (Bellemann et al., 1981; Marsh et al., 1983; Schwartz et al., 1984). At these concentrations DHP could have effects on other membrane currents as well as calcium currents. In the present paper, we examined the effects of nitrendipine on \(Na^{+}\) currents \(I_{Na}\) in cultured cardiac cells from neonatal rat hearts. At micromolar concentrations, the drug blocked \(Na^{+}\) channels. The block was voltage and use dependent, and had characteristics similar to the block of Na+ channels produced by local anesthetics.

Methods

Culture of Neonatal Rat Heart Cells

Primary cardiac cell cultures were prepared from hearts of neonatal rats (1–3 days old) according to a modification of the method of Mark and Strasser (1966). Hearts were removed under sterile conditions and the ventricles were cut into small pieces. The tissue pieces were incubated at 37°C for 5 minutes in \(Ca^{++}\)-free Hanks' solution containing 0.5% trypsin (Sigma T-0134). The supernatant was removed and added to culture medium, DMEM-10 FCS to stop enzyme action. The cell suspensions were seeded on glass cover-slips which were put into 35 mm Falcon...
dishes containing the culture medium. The cultures were kept in an incubator at 37°C in an H2O-saturated, 5% CO2-95% O2 air atmosphere.

**Electrical Recording**

Electrophysiological recordings were made with the whole-cell patch-clamp techniques as described by Hamill et al. (1981) and Fenwick et al. (1982). Patch pipettes (resistances 2-3 MΩ) were prepared as described by Hamill et al. (1981). In some experiments, series resistance (1 ~ MΩ) was compensated electronically by positive current feedback. Transient cancellation was used as described by Hamill et al. (1981). The experimental chamber was placed on a microscope stage, and solutions superfused the 0.2 ml chamber volume at a rate of 2 ml/min by gravity. To suppress outward currents, the pipettes were filled with a Cs⁺-rich solution of the following composition (mM): CsOH, 110; aspartic acid, 110; CsCl, 20; HEPES, 5; ECTA, 3; pH 7.3, with NaOH. Liquid junction potentials (<5 mV) measured between solution and pipette were compensated. To improve voltage control, Na⁺ in the bath was decreased to 60 mM. The calcium solution was compensated. To improve voltage control, Na⁺ in the bath was decreased to 60 mM. The calcium current was suppressed by substitution of Co** for Ca**, in the presence of MgCl₂. Potassium currents were suppressed by replacing K⁺ with Cs⁺ and by adding tetraethylammonium chloride (TEA) and 4-aminopyridine (4-AP) (Isenberg, 1976; Keynon and Gibbons, 1979a, 1979b; Hagiwara and Byerly, 1981; Yatani et al., 1984). The bath solution contained (mM): NaCl, 60; CsCl, 5; TEA, 20; MgCl₂, 2.5; CoCl₂, 2; 4-AP, 5; glucose, 5.5; sucrose, 80; pH 7.4, with HCl. All experiments were performed at room temperature (20-22°C).

The current and voltage recorded in response to command pulses were monitored on a storage oscilloscope, digitized at 2 μsec/point, with a signal averager. The digitized records were stored on a digital tape recorder. Experimental curve fitting was accomplished with a PDP 11/70 computer using a modified Marquardt-Levenberg routine. In Figure 2, Student's t-test was used.

**Drugs Used**

Nitrendipine (3-ethyl-5-methyl-1,4-dihydro-2,6-dimethyl-4-[3-nitrophenyl]-3,5-pyridine carboxylate, and Bay K8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-[2-trifluoromethylphenyl]-pyridine-5-carboxylate) were kindly supplied by Miles Institute for Preclinical Pharmacology. The drugs were dissolved in absolute ethanol. In some experiments, polyethylene glycol 400 was used. The final concentration of bath solvents was held constant at 0.1% for doses of nitrendipine up to 10⁻⁸ M. When nitrendipine was used at 10⁻⁴ M concentration, the solvent concentration rose to 1%.

**Results**

Cardiac myocytes prepared from neonatal rats 1-3 days old are spherical when first dispersed, and have a diameter of 10-20 μm. The geometry is therefore very favorable for voltage clamping. In addition, electron micrographs show that T-tubules are absent (Moses and Kasten, 1979). Electrically, the cells behave as a simple R-C circuit with a time constant <100 μsec (Lacerda et al., in preparation). The patch pipettes had tip resistances of 3 MΩ or less and the input resistance of the cells we used was ~10 GΩ. The peak I Na were always less than 1000 pA, and the effect of the series resistance was therefore very small. The voltage clamp was judged to be adequate when the current records showed no abnormal deflections and the conductance-voltage relation showed the smooth progression illustrated by the current-voltage (I-V) curves (Fig. 1B). The I-V relations retained their shapes when peak I Na differed by as much as 400-500%, which is further evidence that voltage control was satisfactory.

**Effects of Nitrendipine on I-V Relations and Availability of Na Currents**

Figure 1A shows families of I Na obtained from a single spherical cultured neonatal rat cardiac cell, in the absence (a) and presence of nitrendipine 10⁻⁷ M (b), 10⁻⁵ M (c), and 10⁻⁴ M (d). The currents in 1A are produced by depolarizing voltage steps of 10-mV increments from -50 to -20 mV from a holding potential of -80 mV. Records were taken in control solution, and then 5 minutes after exposure to each drug concentration. The 10-msec test pulses were applied at a low frequency (0.1 Hz). The peak I Na was measured as the maximum inward current displacement from the holding current level (zero current level), since the leakage current was negligibly small over the range explored (Fig. 1B). Nitrendipine produced a concentration-dependent decrease in I Na without changing the I-V relations. Reduction of I Na began at concentrations as low as 10⁻⁷ M. I Na threshold and reversal potential (E Na) remained unchanged. There were no changes in the waveform during the voltage clamp step (Fig. 6), and therefore development of block during the voltage step was either too fast or too slow to be detected. As we shall see later, one component of block develops

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very slowly (Figs. 5 and 6). These effects were reversible and the current was restored to its control value after a 5- to 10-minute wash period. The solvent effects were tested and were found to be negligible.

For each concentration of drug, I-V relations before and after application were obtained using the same protocol shown in Figure 1. Percent reductions of peak $I_{Na}$ obtained in solutions containing various concentrations of nitrendipine were collected and are plotted in Figure 2. Nitrendipine produced an inhibition of $I_{Na}$ at concentrations as low as $10^{-7}$ M, which is approximately the concentration required for half-maximal inhibition of resting Ca$^{++}$ channels in cardiac cells (Lee and Tsien, 1983; Brown et al., 1984a; Sanguinetti and Kass, 1984; Bean, 1984). Similar inhibition of $I_{Na}$ is also observed in neonatal rat cells (Yatani et al., in preparation).

A new dihydropyridine derivative (Bay K8644) has recently been shown to be a potent Ca$^{++}$ channel agonist (Brown et al., 1984b), and it is inhibited by nitrendipine (Schramm et al., 1983a). Receptor-binding studies suggest that nitrendipine and other members of the dihydropyridine family bind to the same receptor and the receptor differs from other known receptors such as $\alpha$- and $\beta$-adrenergic receptors (Schramm et al., 1983b).

We therefore examined the action of nitrendipine in combination with Bay K8644 (Fig. 2). The top panel in Figure 2 shows $I_{Na}$ before (a), 5 minutes after $10^{-5}$ M nitrendipine was added (b), then after 5 minutes of exposure to nitrendipine ($10^{-5}$ M) plus $10^{-7}$ M Bay K8644 containing solution (c). Bay K8644 partially reversed the nitrendipine-induced inhibition of $I_{Na}$. In this experiment, the membrane was clamped to $-30$ mV, 30 msec in duration, from a holding potential of $-80$ mV. Bay K8644 ($10^{-7}$ M) alone did not induce a change of $I_{Na}$. At concentrations greater than $10^{-7}$ M, Bay K8644 increased $I_{Na}$ in five of eight experiments. The question concerning a possible interaction of Bay K8644 with the Na$^{+}$ channel is currently being studied.

The block of Na$^{+}$ channels by nitrendipine was partly reversed by shifting the holding potential ($-90$ mV) to more negative values ($-110$ mV) (Fig. 3, inset). Thus, the interaction between nitrendipine block and Na$^{+}$ channel availability was examined over a range of potentials in the absence and presence of the drug. Figure 3 shows a representative steady state inactivation curve of a cultured neonatal rat heart cell. The extent of inactivation of $I_{Na}$ was determined by a double-pulse protocol. The pre-pulse duration was 500 msec, which produces steady state inactivation of $I_{Na}$ under control conditions, and the test potential was $-30$ mV and 4 msec long. The test $I_{Na}$ current produced at the most hyperpolarized prepulse potential ($I_{Na}$, drug) = 764 pA. Inset: (a) $I_{Na}$ elicited by a 4-msec depolarizing pulse to $-30$ mV from a holding potential of $-80$ mV; (b) in the presence of nitrendipine ($10^{-7}$ M), $I_{Na}$ was elicited as in (a), then holding potential was hyperpolarized to $-110$ mV (open circle).

\[
h_{Na} = \frac{1}{1 + \exp(V_m - V_h)/k}
\]

where $V_h$ is the midpotential and $k$ is the slope of the curve. The mean control values for $V_h$ and $k$ in
Nitrendipine shifted the inactivation curve to negative potentials with the same steepness factor as the control. \( I_{\text{Na}} \) was reduced by nitrendipine even with conditioning pulses up to \(-120\ \text{mV}\), although this is not apparent, since the points were normalized. Negative conditioning pulses of more than \(-120\ \text{mV}\) showed no further effect on current size, suggesting that the effect of the holding potential on nitrendipine block may saturate at \(-120\ \text{mV}\). The corresponding \( V_h \) values in the absence and presence of nitrendipine \((10^{-5} \text{ M})\) were \(-82\ \text{mV}\) and \(-89\ \text{mV}\), respectively. The nitrendipine-induced shift of the inactivation curve was larger for high concentrations of the drug, and there was no consistent change in the \( k \) value. The shifts of the inactivation curve in various nitrendipine concentrations and the normalization factor \([I_{\text{Na}}(\text{drugs})/I_{\text{Na}}(\text{control})]\) used for curve fitting are presented in Table 1.

**Use-Dependent Action of Nitrendipine**

Our result showing that nitrendipine shifted the inactivation curve in the hyperpolarizing direction resembled the effects of local anesthetics having a tertiary amine structure, such as lidocaine. According to the guarded gate hypothesis, this is due to retention of the drug by closed \( h \) gates (Starmer et al., 1984). In either case, it was of interest to examine use- or frequency-dependent effects. Thus, trains of 20 depolarizing clamp pulses \((-80\ \text{to}\ -30\ \text{mV}\) for 50 msec) were applied at different pulse intervals, ranging from 1000 to 200 msec (Fig. 4). The cells were rested for 5 minutes before the train of pulses was applied. The top panels in Figure 4 show superimposed \( I_{\text{Na}} \) during the 1st and 20th pulse at a pulse interval of 5 minutes before the train of pulses was applied. The time course of \( I_{\text{Na}} \) is shown in Figure 5A. Recovery at \(-90\ \text{mV}\) was assessed by measuring \( I_{\text{Na}} \) in response to paired 50-msec depolarizing pulses to \(-30\ \text{mV}\), separated by a variable length of time. Each double-pulse sequence was followed by a 10-second rest period. The recovery curve was plotted as the relation of \( I_{\text{Na}}(\text{conditioning})/I_{\text{Na}}(\text{control}) \) against the pulse interval. The same data shown in Figure 5A was plotted semilogarithmically \([\log(1-I_{\text{Na}}/I_{\text{Na}})]\) against the pulse interval in Figure 5B.

In the absence of nitrendipine, the time course was fitted by an exponential with a time constant \( \tau \) of 40 msec. With nitrendipine \((10^{-5} \text{ M})\), the recovery of \( I_{\text{Na}} \) was fitted by two exponentials. The first phase \( \tau_1 \) of 50 msec corresponds to the recovery tau in the absence of drug and its amplitude was reduced about 50%. The second phase was much slower, with \( \tau_2 \) of 220 msec. Similar prolongation of \( I_{\text{Na}} \) recovery was found in five additional experiments. The dependence of test pulse \( I_{\text{Na}} \) on conditioning potential was obtained by the experimental protocol which is identical to that described in Figure 3. Results are expressed as mean ± so; \( n \) = number of experiments.

**TABLE 1**

<table>
<thead>
<tr>
<th>Nitrendipine-Induced Shifts of ( I_{\text{Na}} ) Availability</th>
<th>Normalization factor</th>
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<tr>
<td>[ [\text{Nitrendipine}] ]</td>
<td>[ k ]</td>
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<tr>
<td>[ 10^{-7} ]</td>
<td>[ 7.2 ± 1.0 ]</td>
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<tr>
<td>[ 10^{-4} ]</td>
<td>[ 7.3 ± 0.8 ]</td>
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<td>[ 10^{-3} ]</td>
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**FIGURE 4.** Use-dependent effect of nitrendipine \(10^{-5} \text{ M}\). Panel A: trains of 20 depolarizing pulses \((-80\ \text{to}\ -30\ \text{mV}\) for 50 msec) were applied at an interval of 300 msec. The first and 20th \( I_{\text{Na}} \) were superimposed: (a) control, (b) in the presence of nitrendipine. Panel B: development of use-dependent block of \( I_{\text{Na}} \) in the presence of nitrendipine \((10^{-5} \text{ M})\), at each pulse interval. \( I_{\text{Na}} \) relative to the first pulse was plotted. Voltage clamp steps of 50 msec from \(-80\ \text{mV}\) to \(-30\ \text{mV}\) were applied.
ments with a recovery potential —80 or —100 mV. In each case, the fast tauts were unchanged by the drug, although their amplitudes were reduced. The larger taud had values of 250 ± 30 msec for —80 mV (n = 3) and 180 ± 20 msec for —100 mV (n = 2).

To examine any possible interaction of nitrendipine with Na+ channels when they are in the open state, 20 repetitive depolarizing clamp pulses from —80 to —20 mV, 1 msec in duration, were applied at 5 Hz. This protocol opens the channels but produces little inactivation. The cell was rested for 5 minutes before the test sequence was applied. In the control measurement, the relative I Na did not vary from the first to the 20th pulse. With 10^-5 M nitrendipine, the situation was nearly identical to the control, except that a reduction in the amplitude of the first test pulse of 50% was observed.

In addition, we compared the waveform of I Na during depolarizing voltage pulses before and after exposure to nitrendipine, since drugs that bind to the open state of the channel have been shown to increase the inactivation rate of current through the channels (Coloquhoun and Hawkes, 1983). Figure 6a compares the time course of I Na before and after the application of nitrendipine (10^-5 M). In b, the magnitudes of I Na before and after the drug are normalized. Depolarizing pulses were applied to —10 mV for 5 msec at 0.1 Hz from a holding potential of —80 mV. As shown in Figure 6a, I Na measured in the presence of the drug is reduced; however, the waveform, in particular the rate of inactivation, is not altered (see also Fig. 1).

Discussion

Our results show that the DHP Ca++ channel blocker nitrendipine also blocks Na+ channels. The block occurs at concentrations that are required for full block of I Ca (Lee and Tsien, 1983; Bean, 1984). These concentrations are also within the range of the concentrations at which low-affinity nitrendipine-binding sites in cardiac tissue are occupied (Bellemann et al., 1981; Schwartz et al., 1984). Nitrendipine action on Na+ channels was both voltage and use dependent and the effects are similar to those of another group of Na+ channel blockers, the local anesthetics (Lee et al., 1981; Bean et al., 1983; Sanchez-Chapula et al., 1983). Block to open channels was not observed, but it could have been so fast that a change in waveform might not have been detected. Effects such as those observed presently have been explained by the modulated receptor hypothesis of local anesthetic action (Hille, 1977a; 1977b; Hondeghem and Katzung, 1977, 1984) in which differing drug affinities for different channel conformations are assumed. The guarded gate hypothesis of Starmer et al. (1984) might be as reasonable; in this explanation, relief of block by hyperpolarization is explained by clearance of drug when the closed h gates are reopened. It should be noted that block to inactivated channels developed too slowly to be detected during the voltage clamp step. This is consistent with the recovery results and the superimposable waveforms shown in Figure 6.
Figure 7: Modulated receptor hypothesis for nitrendipine binding to Na⁺ channels. R is rested state; RN is rested state with nitrendipine bound. I and IN are the corresponding forms of the inactivated state. One-to-one drug binding to the Na⁺ channel is assumed, with different affinity for rested (K_R) and inactivated channels (K_I). Binding to open channels is omitted. Drug-associated channels differ from drug-free channels in that they do not conduct. Panel A: dose-response for block at holding potentials of -80 and -120 mV. The data at -80 mV are the same as in Figure 2 (O). At -120 mV, the data were the same as shown in Table 1 (•). Solid curve is best least squares fit to: Relative decrease of
\[ \frac{I}{I_0} = \frac{[M]}{[M] + K_a} \]

where \([M]\) is a drug concentration and \(K_a\) is an apparent dissociation constant. Panel B: half-time of nitrendipine block determined by the same protocol as in inset. Points show mean value for four cells. Inset shows the time course of nitrendipine block with half-time of 1.2 minutes. Test pulse to -20 mV, 10 msec long, was applied from a holding potential of -80 mV at 0.1 Hz. Solid curve is best fit of \(1/(1 + KN(1-h))t_\text{in} = 1.443 t_\text{in} \) where \(t_\text{in}\) is half time, was calculated. \(h\) is the fraction of channels in the rested state and \(N\) is the drug concentration.

Although there is less block at higher concentrations than expected, the curve fits the data at lower concentrations. Following the development used by Bean et al. (1983), the apparent dissociation constant \(K_{a\text{pp}}\) that is determined by a mix of actual binding reactions is
\[ K_{a\text{pp}} = \frac{1}{(h/K_R) + (1-h)/K_I} \]

where \(h\) is the fraction of channels in rested state in the absence of drug, \(K_R\) is the dissociation constant for binding to rested state, and \(K_I\) is the dissociation constant for binding to the inactivated state. The data in Figure 7A are consistent with the predicted form. \(K_R \gg K_I, K_I \approx (1-h)/K_{a\text{pp}}\). In the experiment at -80 mV, \(h\) was about 0.5, and thus the observed apparent dissociation constant of 3 \(\times 10^{-8}\) M would correct to a value of 1.62 \(\times 10^{-4}\) M for binding to the inactivated state. The model in Figure 7 may also be used to evaluate the kinetics with which nitrendipine block developed. Assuming that the equilibration of channels between rested and inactivated states has occurred and that the binding reaction is rate-limiting, nitrendipine block will develop with the time constant of the second-order reaction; \(\tau_{\text{on}} = 1/[k_{-1} + (1-h)N.k_1]\), in which \(N\) is the nitrendipine concentration, \(k_1\) the forward-rate constant, and \(k_{-1}\) the off-rate constant. The ratio \(k_1/k_{-1}\) is determined from the fit to the equilibrium dose-response curve. The expression for the expected time constant can then be fit to the experimental data with \(k_{-1}\) as a free parameter (Bean, 1984).
As Figure 7B shows, the model accurately predicts the form of experimental data, and a value of 0.0002/sec is obtained for $k_{-i}$, which is roughly equal to the off-rate constant for the high-affinity nitrendipine-binding site for cardiac membrane (Janis et al., 1984). The calculated on-rate constant for the IC$_{50}$ effect is then two to three orders smaller than the on-rate constant for the high-affinity site. These results may be underestimates, however, since the values of hyperpolarizing shifts in the availability curve for IC$_{50}$ that we have observed are underestimates. As Figure 7B shows, the prepulse duration should have been minutes, but such durations were not experimentally feasible. It should also be noted that use dependence of Ca$^{++}$ channel block occurs, but seems not to be as prominent as the effect on Na$^{+}$ channels (Sanguinetti and Kass, 1984; Bean, 1984; Yatani et al., in preparation).

These observations, and the interaction with Bay K8644, constitute strong evidence that the effects on the Na$^{+}$ channels are due to an interaction of DHP with the Na$^{+}$ channel itself. It seems quite unlikely that the voltage-dependent effects on the Na$^{+}$ channels could be due only to absorption of DHP by membrane lipids. The fact that a component of the early phase of recovery of $I_{Na}$ from inactivation persisted at very large concentrations also argues for a specific action of the drug on Na$^{+}$ channels. This has been confirmed in single-channel experiments (Kunze, Yatani, and Brown, manuscript in preparation). In this regard, it should be noted that Bay K8644 has a specific action on open Ca$^{++}$ channels at concentrations of $10^{-5}$ M (Hess et al., 1984; Brown et al., 1984b). Nitrendipine also acts on Ca$^{++}$ channels via the low-affinity sites (Yatani et al., unpublished work). It should be noted that, since Na$^{+}$ and Ca$^{++}$ channels have a different voltage dependence of inactivation, there is a potential range (about $-70$ mV) in which most Na$^{+}$ channels are inactivated but very few Ca$^{++}$ channels are inactivated. At these potentials, block of Na$^{+}$ channels by nitrendipine would be comparable to block of Ca$^{++}$ channels. Thus nitrendipine will not be a selective blocker in all instances.

It has been shown that cardiac sarcolemmal vesicles have two saturable binding sites for DHP, one with a $K_d$ of a fraction of a nanomolar, the other with a $K_d$ of a fraction of a micromolar (Bellemann et al., 1981; Schwartz et al., 1984). The number of high-affinity sites is in reasonable agreement with estimates of Ca$^{++}$ channel density (Yatani et al., unpublished work), but the number of low-affinity sites is one or more orders larger. The Na$^{+}$ channels may be included among these lower affinity sites. The low-affinity sites may also include the adenosine transporter (Glassmann, 1985), and this raises the possibility that effects of DHP on Na$^{+}$ channels could be secondary to effects on this molecule. It appears likely that the present results can be extended to the DHP class of Ca$^{++}$ channel agonists such as Bay K8644. The fact that nitrendipine's effects were reduced by Bay K8644 would support the idea that DHP with Ca$^{++}$ agonist actions bind to Na$^{+}$ channels, as well. The significance of these findings awaits further study, but suggests that Na$^{+}$ and Ca$^{++}$ channels may contain some common structural features which operate in both types of channels. A similar conclusion could be drawn from the study of Romey and Lazdunski (1982).

The present results clearly have practical consequences. High doses of DHP Ca$^{++}$ channel antagonists will have actions on cardiac $I_{Na}$, in the heart, and the effects will be use-dependent. This is important clinically, since arrhythmias frequently involve cardiac tissue that is partially depolarized or is discharging at a fast rate.

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Yatani and Brown/Nitrendipine and Cardiac Sodium Currents


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