

Block of T-Type Ca Channels in Guinea Pig Atrial Cells by Antiarrhythmic Agents and Ca Channel Antagonists

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ABSTRACT Myocardial cells have two types of Ca channels commonly called T-type and L-type. Whole cell Ca channel currents in guinea pig atrial myocytes can be separated and quantitated by analyzing channel closing kinetics after a brief depolarization (tail current analysis). L-type Ca channels deactivate rapidly when the membrane is repolarized and T-type Ca channels deactivate relatively slowly. Ca channel block by the therapeutically useful Ca channel antagonists is voltage dependent, so it is desirable to study block of both channel types over an extended voltage range. Tail current analysis allows this and was used to study block of both types of Ca channels under identical conditions. Amiodarone, bepridil, and cinnarizine block T-type Ca channels more potently than L-type Ca channels when binding equilibrates at normal diastolic potentials (~ -90 mV). None of these drugs is a selective blocker of T-type Ca channels because block of L-type Ca channels is enhanced when cells are almost completely depolarized. Although weak block of T-type Ca channels by 1,4-dihydropyridines has usually been reported, we found that felodipine blocks these channels with high affinity. When most T-type Ca channels are inactivated, the apparent dissociation constant (K_I) is 13 nM. Felodipine also blocks T-type Ca channels in GH₃ cells (a cell line derived from rat anterior pituitary), but $K_I = 700$ nM. Thus, T-type Ca channels in different cell types are pharmacologically distinct. Felodipine can block L-type Ca channels in atrial cells more potently than T-type Ca channels, but block of L-type Ca channels is potent only at depolarized potentials; block of both channel types is comparable at normal diastolic membrane potentials. Felodipine and the 1,4-dihydropyridines isradipine and (-)-202-791 are approximately equipotent at blocking T-type Ca channels, but differ substantially in potency for block of L-type Ca channels. Block of T-type Ca channels may account for some of the pharmacological effects of 1,4-dihydropyridines and for the antiarrhythmic activity of amiodarone and bepridil.

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INTRODUCTION

Myocardial cells have predominantly two populations of Ca channels, called T-type (or low voltage activated) and L-type (or high voltage activated) (Bean, 1989). The pharmacological properties of the T-type Ca channels are not well characterized because this channel type was discovered only recently. There are several reasons for believing that blockers of T-type Ca channels would be novel and useful antiarrhythmic agents: (a) T-type Ca channels are prevalent in cells that can display pacemaker electrical activity, such as the sino-atrial node and Purkinje fibers (Hagiwara, Irisawa, and Kameyama, 1988; Hirano, Fozzard, and January, 1989; Tseng and Boyden, 1989). Current through these channels strongly affects rates of rhythmic activity because T-type Ca channels activate substantially in the voltage range where pacemaker depolarization occurs. Hence, blockers of T-type Ca channels should be efficacious in treating some forms of supraventricular tachycardia. (b) None of the negative feedback mechanisms that apply to L-type Ca channels apply to T-type Ca channels (Fedulova, Kostyuk, and Veselovsky, 1985; Carbone and Lux, 1987), so that blockers of T-type Ca channels may have the greatest effect on Ca influx in cells that are metabolically compromised. L-type Ca channels are subject to a number of negative feedback mechanisms that can inhibit Ca overload. These channels are subject to Ca-induced inactivation, whereby increases in cell Ca inhibit further Ca entry (Pelzer, Pelzer, and McDonald, 1990). Activation of L-type Ca channels is modulated by a number of intracellular metabolites such as ATP, so that Ca influx through L-type Ca channels will be reduced in metabolically compromised tissue (Pelzer et al., 1990). Blockers of T-type Ca channels may inhibit arrhythmias arising from Ca overload with relatively little negative inotropic effect. (c) The therapeutically useful Ca entry blockers are thought to selectively block L-type Ca channels (Bean, 1985; Nilius, Hess, Lansman, and Tsien, 1985; Mitra and Morad, 1986; Hagiwara et al., 1988; Tytgat, Vereecke, and Carmeliet, 1988; Hirano et al., 1989; Tseng and Boyden, 1989; Bois and Lenfant, 1991; but see Akaike, Kanaide, Kuga, Nakamura, Sadoshima, and Tomoike, 1989a; Kuga, Sadoshima, Tomoike, Kanaide, Akaike, and Nakamura, 1990). To our knowledge, no selective blocker of the T-type Ca channels has been identified.

We have evaluated the effects of several known antiarrhythmic agents and Ca channel antagonists on T-type Ca channels. Our goal was to determine whether block of T-type Ca channels could account for antiarrhythmic activity and to identify structural leads in a search for a selective blocker of T-type Ca channels. Since most of the drugs studied cause voltage-dependent block of L-type Ca channels, it was highly desirable to utilize techniques that would allow us to study both L- and T-type Ca channels over a broad range of voltages and to reliably separate these two components of Ca channel current. As in some other types of excitable cells, T-type Ca channels in atrial myocytes deactivate much more slowly than L-type Ca channels, so total Ca channel current can be separated into two components by tail current analysis. We unexpectedly found that the slow component of Ca channel tail current was potently blocked by some 1,4-dihydropyridines such as felodipine, which were widely believed to be specific blockers of L-type Ca channels (Van Skiver, Spires, and Cohen, 1989). This finding generated concern that some L-type Ca channels

deactivate slowly in atrial myocytes, so that tail current analysis could not be reliably used in myocardial cells to separate the two components of Ca channel current. Consequently, we made a detailed study of the ionic selectivity and susceptibility to block by protons of both components of tail current.

The first part of the Results section will show that tail current analysis can be used to separate and quantitate current through two types of Ca channels in guinea pig atrial myocytes. The second part of the Results section will characterize the block of both types of Ca channels by commonly used antiarrhythmic agents and Ca channel antagonists.

Preliminary reports of parts of this work have been published (Van Skiver, Spires, and Cohen, 1988; Van Skiver et al., 1989).

METHODS

Preparations

Ca channel currents were measured in guinea pig atrial cells using the whole cell variation of the patch voltage clamp technique. The enzymatic dissociation procedure was similar to that described by Mitra and Morad (1985), except that cells were placed into tissue culture media at 37°C for at least 1 h before use. We chose atrial cells rather than ventricular cells for our studies for three reasons: (a) previous studies indicated that the ratio of T-type Ca channels to L-type Ca channels might be higher in atrial cells (Bean, 1985); (b) atrial cells are better suited to the whole cell variation of the patch voltage clamp technique because of their smaller size and more favorable shape; and (c) adult atrial cells have been successfully maintained in tissue culture, so that cells can be used for several days after isolation (Bechem, Pott, and Rennebaum, 1983). Almost all studies of T-type Ca channels were performed on the same day that cells were dissociated because the current through T-type Ca channels seemed to decrease with time in culture.

Voltage Clamp Measurements

The methods used for patch voltage clamp experiments in our laboratory have been described previously (Cohen and McCarthy, 1987). An Axopatch 1A amplifier (Axon Instruments, Inc., Foster City, CA) was modified to allow low pass filtering of the series resistance compensation and a headstage with a 50-M Ω feedback resistor was used (model CV 3-0.1/100; Axon Instruments, Inc.). Membrane current was sampled at 40 or 50 kHz and filtered with a 4-pole Bessel filter with a cut-off frequency (-3 db) of 10 or 20 kHz. Linear leak and capacity currents were subtracted digitally by scaling the response to a test pulse from -100 to -140 mV. Bath and pipette solutions were chosen so that tail currents were typically 0.5-2 nA in amplitude. Larger tail currents were avoided in order to limit errors due to incomplete series resistance compensation. Smaller currents were avoided to minimize the contribution of dihydropyridine-sensitive gating currents to tail current measurements (see below).

Data Analysis

Each tail current measurement was fit by the sum of two exponentials plus a constant using the Levenberg-Marquardt nonlinear least-squares curve-fitting procedure (Press, Flannery, Teukolsky, and Vetterling, 1986). The curve-fitting procedure excluded data collected before the change in membrane voltage was complete (typically 100-200 μ s after a step in command voltage). The reported amplitudes of the exponentials represent the amplitude at the end of this blanking period and current recordings in the figures also exclude these data.

The steady-state availability of each component of Ca channel current was determined by plotting the amplitude of each component of tail current vs. the prepulse potential. The prepulse duration was typically 30 s. Each data set was fit by a two-state Boltzmann distribution, given by $I = I_{\max}/[1 + \exp [(V_p - V_{1/2})/k]]$, where V_p is the prepulse potential, k is the slope factor, and $V_{1/2}$ is the potential corresponding to half-maximal current. Binding constants for rested and inactivated states of the Ca channels were calculated by use of the modulated receptor theory (Bean, 1984; Hondeghem and Katzung, 1984; Sanguinetti and Kass, 1984). Binding constants of drug for the rested and inactivated states (denoted by K_R and K_I , respectively) were calculated using the equation $K_I = D/[(1 + D/K_R) \exp(-\Delta V/k) - 1]$, where D is the drug concentration and ΔV is the shift in the availability curve (Bean, Cohen, and Tsien, 1983). K_R was calculated from the amount of block at $V_p = -110$ mV.

Our analysis of block of L-type Ca channels assumes that channel block is proportional to block of the rapidly decaying component of tail current. There are two major concerns with this analysis: (a) the time constant describing tail current decay (≈ 300 μ s at -45 mV) is not much slower than the settling time of the voltage clamp after a step in voltage; and (b) dihydropyridine-sensitive gating currents decay at about the same rate as L-type Ca channel tail currents and can account for a signal of ~ 100 pA in atrial cells (Field, Hill, and Lamb, 1988; Bean and Rios, 1989; Hadley and Lederer, 1989, 1991). The contribution of gating currents to tail current measurements depends on the nature and concentration of charge carrier and on the pulse protocol. The validity of our measurements of L-type currents by tail current analysis was assessed by comparing the current during a test pulse elicited from a holding potential ≥ -45 mV with the tail current measured after repolarization. Both measures indicated the same time course and voltage dependence of channel inactivation and the same effect of dihydropyridines on steady-state availability. Apparently both ionic current and gating current are similarly affected by the drugs that we have studied.

Solutions and Drugs

The bath and pipette solutions used in electrophysiological experiments were designed to minimize currents through Na and K channels and run-down of L-type Ca currents. The pipette solution usually contained (mM): 87 *N*-methyl-D-glucamine (NMG) glutamate, 20 NMG-F, 20 NMG-Cl, 1 tetrabutylammonium Cl, 11 BAPTA, 0.9 CaCl₂, 1 MgCl₂, 20 HEPES, 5 Mg-ATP, and 0.1 Li₂GTP, pH 7.2 with CsOH. In later experiments, Cs salts were used in place of NMG. F⁻ enhanced the L-type Ca channel currents. The bath solution for most experiments contained (mM): 134 tetraethylammonium (TEA) Cl, 20 BaCl₂ or CaCl₂, 0.5 MgCl₂, and 10 HEPES, pH 7.5 with CsOH. Solutions with a lower concentration of charge carrier were made by mixing the standard bath solution with a similar solution containing 165 mM TEA-Cl and no Ba or Ca. Solutions with pH 9.8 contained 10 mM 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS) in place of 10 mM HEPES. Solutions were gassed with 100% O₂ and experiments were conducted at room temperature (18–24°C).

RESULTS

Figs. 1–4 present a characterization of the components of Ca channel tail current. Tail currents are measured when the membrane is repolarized from a depolarizing pulse that activates channels. Their time course usually indicates the rate of channel closing (deactivation). Guinea pig atrial cells have two populations of Ca channels that can be distinguished by their rate of deactivation. As in some other types of excitable cells, the T-type Ca channels deactivate slowly when the membrane is repolarized and the L-type Ca channels deactivate rapidly (Cota, 1986; Matteson and

Armstrong, 1986; Carbone and Lux, 1987; Cohen, McCarthy, Barrett, and Rasmussen, 1988; Hiriart and Matteson, 1988; Kostyuk and Shirokov, 1989; McCarthy and Cohen, 1989).

Fig. 1 shows that atrial cells have two components of tail current that differ in the voltage dependence of inactivation. The left panel shows superimposed recordings of Ca channel currents for prepulse potentials (V_p) of -90 , -30 , and 0 mV. The pulse protocol is shown in the inset of the right panel. When $V_p = -30$ mV, the current deactivated rapidly and monoexponentially after a brief test pulse ($\tau = 0.30$ ms).

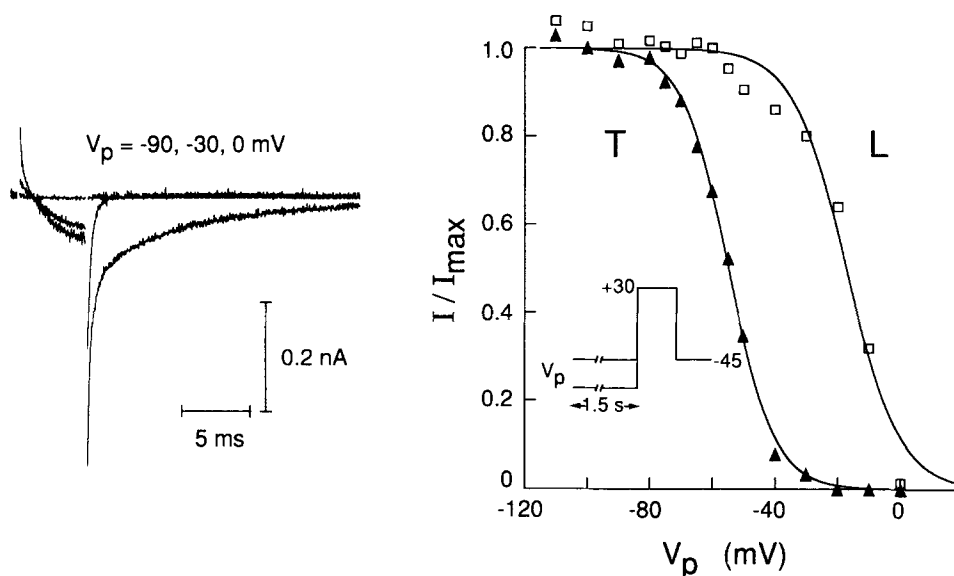


FIGURE 1. The two components of tail current through Ca channels differ in the voltage dependence of inactivation. (Left) Superimposed current records measured during a 5-ms test pulse to $+30$ mV and after repolarization to -45 mV for the indicated prepulse potentials (V_p). The charge carrier was 20 mM Ca. (Right) The relative amplitude of each component of tail current vs. V_p . The solid curves indicate the best fit by a two-state Boltzmann distribution. For the slowly decaying component of tail current (indicated by filled triangles and the letter T), $I_{\max} = 144$ pA, $V_{1/2} = -54.9$ mV, and $k = 7.3$. For the rapidly decaying component of tail current (indicated by open squares and the letter L), $I_{\max} = 257$ pA, $V_{1/2} = -17.3$ mV, and $k = 8.5$. The inset shows the pulse protocol used. See text for further details.

When $V_p = -90$ mV, the tail current was biexponential. The fast component had the same time constant as seen for $V_p = -30$ mV and nearly the same amplitude, but a slowly deactivating component of current was also evident that decayed with $\tau = 6.07$ ms. When $V_p = 0$ mV, both components of Ca channel current were completely inactivated. Each tail current measurement was fit by the sum of two exponentials plus a constant. The right panel of Fig. 1 shows the relative amplitude of each exponential as a function of prepulse potential. The open squares indicate the amplitude of the rapidly decaying exponential and the filled triangles indicate the

amplitude of the slowly decaying exponential. The solid curve through each data set is the best fit by a two-state Boltzmann distribution. The main point here is that the two components of tail current differ in the voltage dependence of inactivation and the slowly deactivating current inactivates at more negative potentials.

Fig. 2 shows the use of tail current analysis to determine the voltage dependence of activation of each component of Ca channel current. The inset of the right panel shows the pulse protocol used to measure the voltage dependence of activation. The left panel shows superimposed recordings of Ca channel currents for the indicated test potentials (V_t). Larger depolarizations elicited tail currents of greater amplitude.

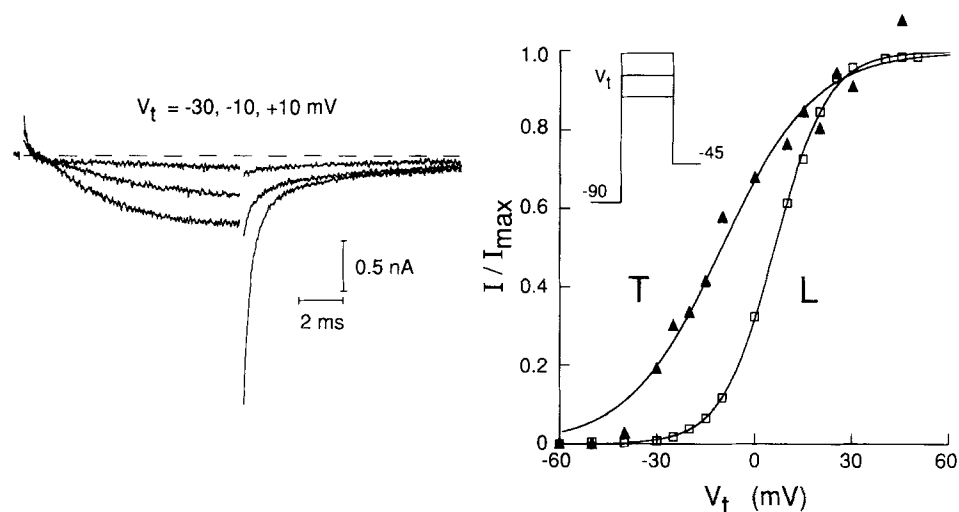


FIGURE 2. The two components of tail current through Ca channels differ in the voltage dependence of activation. (*Left*) Superimposed current records measured during a 10-ms test pulse to the indicated test potential (V_t) and after repolarization to -45 mV. The charge carrier was 5 mM Ba. (*Right*) The relative amplitude of each component of tail current vs. V_t . The solid curve through each set of data indicates the best fit by the equation $I/I_{\max} = [1 + \exp\{(V_m - V_t)/k\}]^{-1}$. For the slowly decaying component of tail current (indicated by filled triangles), $I_{\max} = 403$ pA, $V_m = -10.2$ mV, and $k = 14.2$. For the rapidly decaying component of tail current (indicated by open squares), $I_{\max} = 1,745$ pA, $V_m = +6.2$ mV, and $k = 8.1$. The inset shows the pulse protocol used. See text for further details.

The right panel shows that the components of tail current differ in the voltage dependence of activation. The relative amplitude of each component of tail current is plotted vs. test potential (V_t). The open squares indicate the normalized amplitude of the rapidly decaying exponential and the filled triangles indicate the normalized amplitude of the slowly decaying exponential. The slowly deactivating current activates at more negative test potentials, indicating that this current is “low voltage activated” or T-type.

We observed an extra component of Ca channel current during the test depolarization when the holding potential was changed from -45 to -90 mV. As expected

from previous studies of T-type Ca channels in myocardial cells, this extra current component inactivates rapidly and activates at more negative potentials than the slowly inactivating current (Bean, 1985; Nilius et al., 1985; Mitra and Morad, 1986; Bonvallet, 1987; Hagiwara et al., 1988; Droogmans and Nilius, 1989; Hirano et al., 1989; Kawano and DeHaan, 1989; Tseng and Boyden, 1989; Tytgat, Nilius, and Carmeliet, 1990; Xu and Best, 1990; Bois and Lenfant, 1991). The slowly deactivating Ca channel current had the same voltage and time dependence of activation and inactivation as the transient component of inward current observed during long test pulses (data not shown).

The results presented thus far are consistent with the idea that the two components of tail current arise from two distinct populations of Ca channels, but they do not rule out the possibility that some L-type Ca channels deactivate slowly and contribute to the slow component of tail current. Several laboratories have reported that L-type Ca channels sometimes shift into an alternate "mode" of gating, characterized by long open times and referred to as "mode 2" (Hess, Lansman, and Tsien, 1984; Pietrobon and Hess, 1990; Yue, Herzig, and Marban, 1990). L-type Ca channels in mode 2 would contribute to a slow component of tail current. However, channels in mode 2 have the same conductance as channels that gate normally and induction of mode 2 activity by BAY K 8644 has little or no effect on ionic selectivity (Hess, Lansman, and Tsien, 1986; but see Lacerda and Brown, 1989). We therefore tested for slowly deactivating L-type Ca channels by characterizing the ionic selectivity of each component of tail current. Figs. 3 and 4 show that the two components of tail current differ in relative current-carrying ability for Ca and Ba. The left-hand panels in Fig. 3 show superimposed current measurements in 5 mM Ca and 5 mM Ba at test potentials of -20 and 0 mV. For $V_t = -20$ mV, the slow component of tail current is decreased in amplitude when Ba is substituted for Ca, while the fast component of tail current is increased. The effect of substituting 5 mM Ba for 5 mM Ca on the slowly decaying component of tail current was evaluated in five experiments; the average decrease after a test pulse to -20 mV was $34.7 \pm 3.0\%$ (\pm SEM, $n = 5$). For $V_t = 0$ mV, substituting Ba for Ca results in an increase in the fast component of tail current with little change in the slow component of tail current. These observations are most readily accounted for by associating each component of tail current with a different channel type. The right-hand panels of Fig. 3 indicate the effect of substituting the charge carrier on the voltage dependence of activation of each component of tail current. As in Fig. 2, the amplitude of each component of tail current is plotted vs. the test potential, and the solid curve through each set of data points is defined by a two-state Boltzmann distribution. If current through L-type Ca channels accounted for a significant portion of the slowly decaying tail current, then this component would be increased in amplitude by substitution of Ba for Ca as charge carrier. No such increase was observed, even for very positive test pulses that are most likely to elicit mode 2 activity. Also note that substituting Ba for Ca as the charge carrier shifts the voltage dependence of activation of T-type Ca channels to more positive potentials, while shifting the activation of L-type Ca channels to more negative potentials. The effect of charge carrier on the voltage dependence of activation of T-type Ca channels can account for the apparent conflict between previous studies of divalent cation permeability (Bean, 1989); depending on the test

potential chosen, substituting Ba for Ca can either decrease the T-type Ca channel current or have no effect.

Several studies have concluded that Ba is more permeable than Ca through L-type Ca channels, but not through T-type Ca channels (Bean, 1985; Nilius et al., 1985;

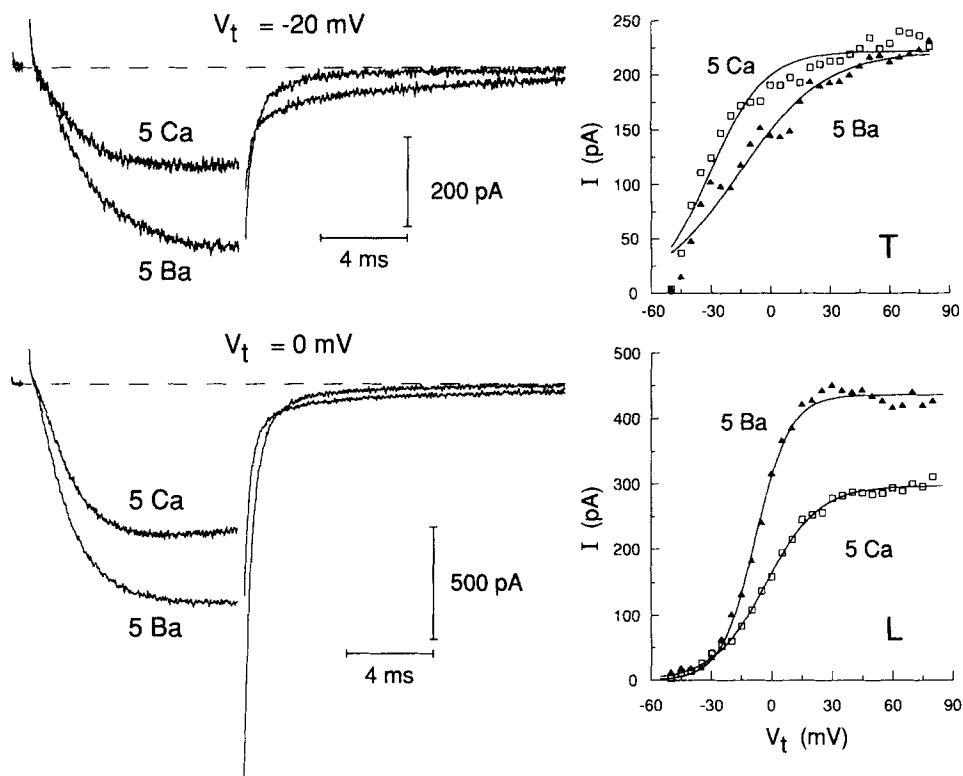


FIGURE 3. The two components of tail current through Ca channels differ in ionic selectivity. (Left) Superimposed current records measured during a 10-ms test pulse to the indicated test potential and after repolarization to -45 mV. The holding potential was -90 mV. (Right) The relative amplitude of each component of tail current vs. V_t with 5 mM Ca or 5 mM Ba as charge carrier. The data are from a different cell than that used for the left panels. The tail currents were fit by the sum of two exponentials plus a constant with time constants of 0.30 and 3.66 ms when Ca was the charge carrier and with time constants of 0.31 and 3.38 ms when Ba was the charge carrier. The solid curve through each set of data indicates the best fit by the equation $I/I_{\max} = \{1 + \exp[(V_m - V_t]/k)\}^{-1}$. For the T-type Ca channels, $I_{\max} = 222$ pA, $V_m = -30.4$ mV, and $k = 13.74$ in 5 mM Ca and $I_{\max} = 221$ pA, $V_m = -15.7$ mV, and $k = 21.50$ in 5 mM Ba. For the L-type Ca channels, $I_{\max} = 297$ pA, $V_m = -2.9$ mV, and $k = 13.24$ in 5 mM Ca and $I_{\max} = 437$ pA, $V_m = -8.1$ mV, and $k = 8.62$ in 5 mM Ba.

Hagiwara et al., 1988; Hirano et al., 1989; Tseng and Boyden, 1989; Bois and Lenfant, 1991). Fig. 4 shows that this conclusion is not generally true, so that the relative amplitude of Ba and Ca currents cannot be used as a criterion for identifying a Ca current as L-type or T-type. Fig. 4A shows the effect of substituting 20 mM Ca

for 20 mM Ba. The record shows superimposed tail current measurements made after a test pulse to +40 mV, so that channel activation was maximal. The arrow indicates the amplitude of the tail current in 20 mM Ca and the inset shows these tail currents on an expanded time scale. As expected, the slow component of tail current is the same in both solutions and the fast component of tail current is larger when Ba is the charge carrier. However, the relative current-carrying ability of Ca and Ba through L-type Ca channels is dependent on the concentration of charge carrier used. At physiological concentrations of charge carrier, Ca currents are larger than

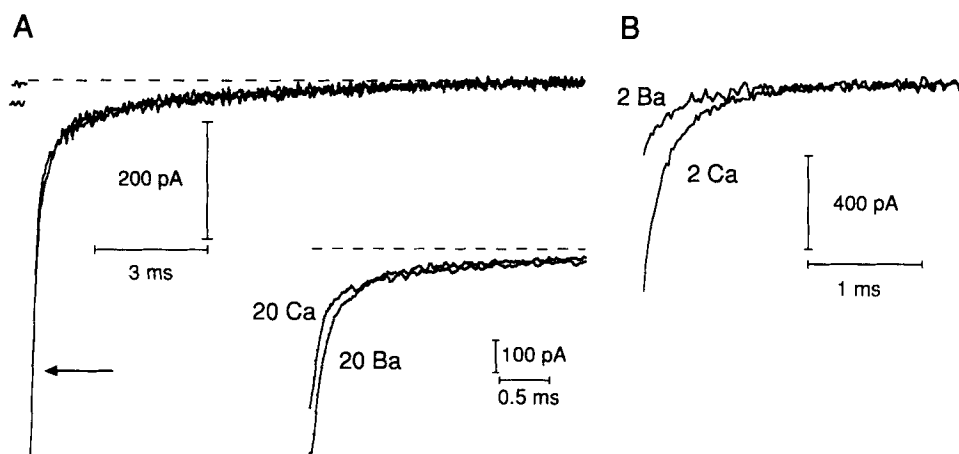


FIGURE 4. The relative selectivity of Ca channels for Ca vs. Ba varies with the concentration of charge carrier. (A) Superimposed tail current records measured with 20 mM Ca or 20 mM Ba as charge carrier. The holding potential was -90 mV, the test pulse was to $+40$ mV for 10 ms, and tail currents were measured at -45 mV. The inset shows the same records on an expanded time scale. Each tail current measurement was fit by the sum of two exponentials plus a constant. For the record in 20 mM Ba, the time constants of decay were 0.21 and 3.22 ms and the amplitudes of the exponentials were 384. and 94. pA, respectively. For the record in 20 mM Ca, the time constants of decay were 0.22 and 4.87 ms and the amplitudes of the exponentials were 220 and 98 pA, respectively. (B) Superimposed tail current records measured with 2 mM Ca or 2 mM Ba as charge carrier. The holding potential was -45 mV, so that T-type Ca channels were not available to open. The test pulse was to $+30$ mV for 5 ms and the membrane was repolarized to -55 mV. Each tail current measurement was fit by a single exponential. In 2 mM Ba the amplitude was 253 pA and the time constant of decay was 0.41 ms. In 2 mM Ca the amplitude was 696 pA and the time constant of decay was 0.33 ms.

Ba currents (Fig. 4 B). Tail currents after a test pulse to $+30$ mV from a holding potential of -45 mV are shown. The pulse protocol was chosen so that only L-type Ca channels would be available to open during the test pulse (see Fig. 1) and activation of L-type Ca channels would be maximal. The concentration dependence of relative conductance for Ba and Ca is expected from previous studies which approximated ionic transport in terms of Michaelis-Menten reaction kinetics. The V_{\max} for Ca is less than that for Ba, but the K_m for Ca is also less than that for Ba. At low concentrations of charge carrier, Ca currents will be larger than Ba currents, but

at concentrations $\gg K_m$, Ba currents will be larger than Ca currents. The relative conductance for Ba and Ca is also determined by the dependence of open state probability on charge carrier (Cavalie, Ochi, Pelzer, and Trautwein, 1983) and may be modified by competition with Mg or TEA in the bath.

Fig. 5 shows that the two components of tail current differ in susceptibility to block by protons. The effect of increasing bath pH from 7.5 to 9.8 is shown. The top panel shows superimposed current recordings for $V_t = -20$ mV. Raising bath pH to 9.8

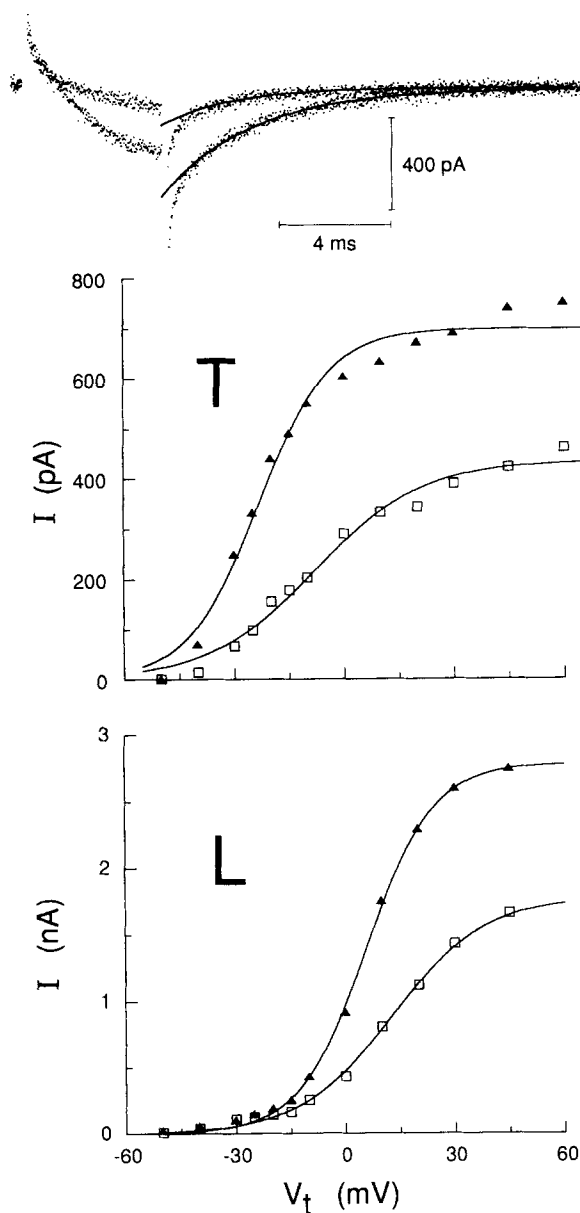


FIGURE 5. The two components of tail current through Ca channels differ in response to changes in bath pH. (Top) Superimposed current records measured during a 5-ms test pulse to -20 mV from -90 mV and after repolarization to -55 mV in bath solutions of pH 7.5 and 9.8. The charge carrier was 5 mM Ba. The solid curve through each current record indicates the best fit of the slowly decaying component of tail current by a single exponential. The time constant was 2.5 ms for pH_o 7.5 and 3.0 ms for pH_o 9.8. The time constant for the rapidly decaying component of tail current was 0.20 ms at both pH's. (Middle) The relative amplitude of the slowly decaying component of tail current vs. V_t . The solid curve through each set of data indicates the best fit by the equation $I/I_{\max} = [1 + \exp[(V_m - V_t)/k]]^{-1}$. For pH_o 9.8 (indicated by filled triangles), $I_{\max} = 700$ pA, $V_m = -23.3$ mV, and $k = 9.71$. For pH_o 7.5 (indicated by open squares), $I_{\max} = 435$ pA, $V_m = -8.0$ mV, and $k = 14.7$. (Bottom) The relative amplitude of the rapidly decaying component of tail current vs. V_t . For pH_o 9.8, $I_{\max} = 2,781$ pA, $V_m = +5.7$ mV, and $k = 9.21$. For pH_o 7.5, $I_{\max} = 1,774$ pA, $V_m = +12.7$ mV, and $k = 12.7$.

preferentially increased the slow component of tail current. The effect of pH on the voltage dependence of activation of both components of tail current is shown in the middle and bottom panels of Fig. 5. As for Figs. 2 and 3, the amplitude of each component of tail current is plotted vs. test potential (V_t). Both components of tail current increase with pH at all potentials, but the voltage dependence of activation is shifted to more negative potentials only for T-type Ca channels. At high pH there is a greater separation of the two components of Ca channel current into low voltage-activated and high voltage-activated components. Similar proton block of T-type Ca channels in guinea pig ventricular cells has been reported (Tytgat et al., 1990).

The results presented thus far support the idea that L- and T-type Ca channel currents in atrial cells can be separated and quantitated by the use of tail current analysis. Tail current analysis allows the study of both types of Ca channels over a broad range of voltages and simultaneous evaluation of block of L- and T-type Ca channels. These are important advantages for pharmacological studies because Ca channel block by the therapeutically useful Ca antagonists is voltage dependent. It is therefore desirable to evaluate the affinity of drugs for the two types of Ca channels using identical pulse protocols.

Fig. 6 shows the effect of amiodarone, bepridil, and cinnarizine on L- and T-type Ca channels. All three drugs have anti-arrhythmic activity and block of L-type Ca channels is thought to contribute to this activity (Van Neuten and Janssen, 1973; Spedding, 1982; Marshall, Winslow, Lamar, and Apoil, 1984; Yatani, Brown, and Schwartz, 1986; Nishimura, Follmer, and Singer, 1989). The top panel of each column shows superimposed measurements of Ca channel currents made with and without the indicated concentration of drug. Only tail currents are shown for the experiments with amiodarone and cinnarizine. The records in drug are indicated by asterisks and the amplitude of the tail current in drug is indicated by an arrow when necessary. Each of these panels shows conditions under which block of T-type channels relative to block of L-type channels is maximal. For these conditions, amiodarone and cinnarizine cause substantial block of T-type Ca channels with little or no block of L-type Ca channels. Drug binding was allowed to equilibrate at the indicated prepulse potential (V_p) and a brief test pulse was applied to activate both L- and T-type Ca channels. The two panels under each set of current records show the steady-state voltage dependence of block of T- and L-type Ca channels determined with the same pulse protocol described in Fig. 1. The middle row of panels shows the amplitude of the slowly decaying component of tail current as a function of V_p . Likewise, the bottom row of panels shows the amplitude of the rapidly decaying component of tail current vs. V_p . The solid curve through each set of data points is a best fit by a two-state Boltzmann distribution and defines channel availability.

Block of T-type Ca channels by amiodarone or cinnarizine is weakly voltage dependent, such that block is enhanced when drug binding equilibrates at less negative prepulse potentials. As in previous pharmacological studies of Na channels and L-type Ca channels, voltage-dependent block of ion channels by neutral molecules can be accounted for by the modulated receptor theory. The solid curve determined by the control data in each panel describes the voltage dependence of the equilibrium between the rested and inactivated states, denoted by R and I,

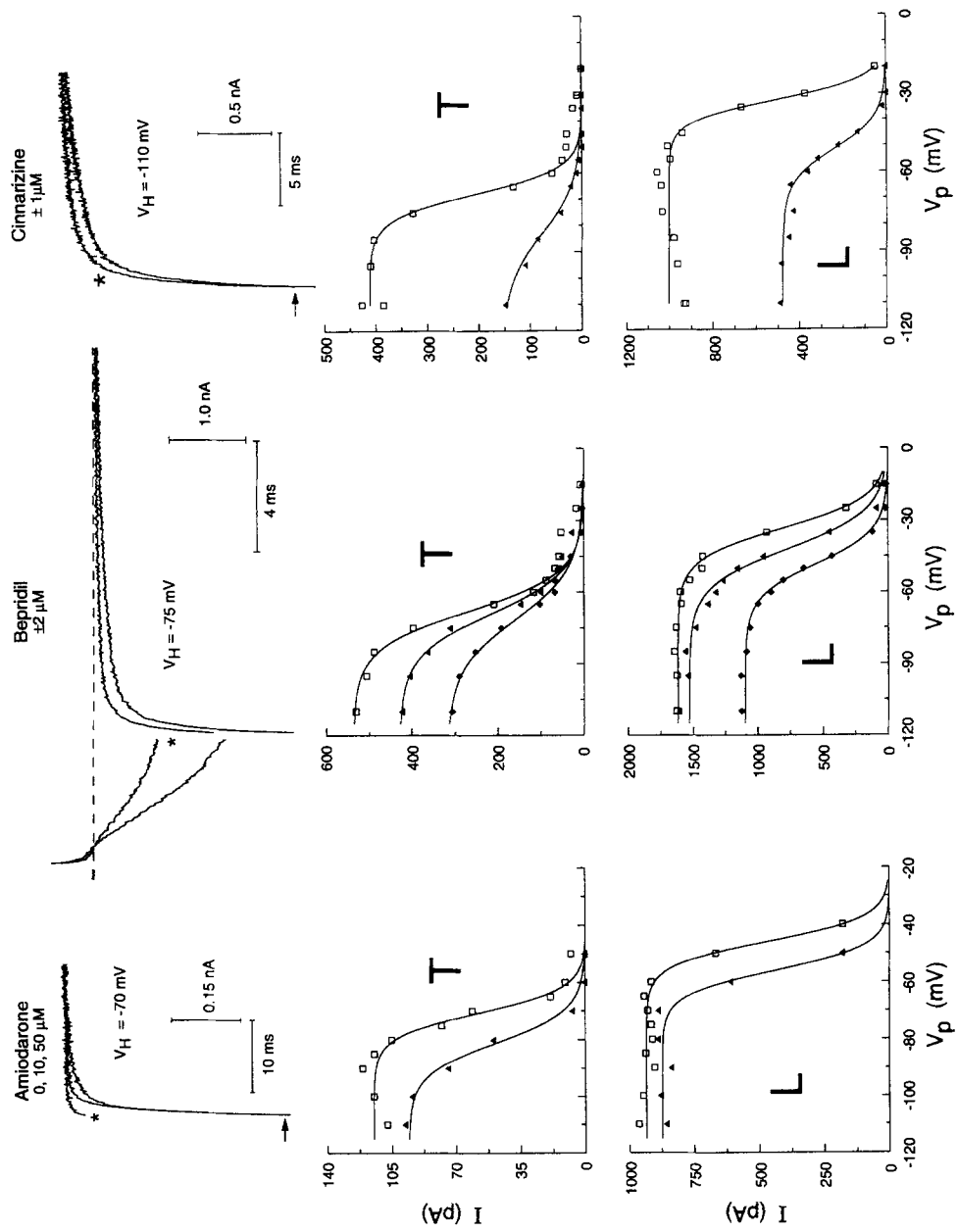


FIGURE 6.

respectively. Depolarization increases the fraction of channels that are inactivated and each drug is postulated to bind to the inactivated state with greater affinity. The data shown in each panel can be used to calculate the binding constants of each drug for the rested and inactivated states, denoted by K_R and K_I , respectively (see Methods). The model predicts both the voltage and concentration dependence of channel block.

In 10 μM amiodarone there was little or no block of the L-type Ca channels but substantial block of the T-type Ca channels when drug binding equilibrated at ≤ -70 mV. It was very difficult to quantitate the block by amiodarone because the block increased steadily during exposure to drug, so that our analysis underestimates the potency of Ca channel block. For the experiment shown, $K_R = 50.7 \mu\text{M}$ and $K_I = 1.16 \mu\text{M}$ for block of the T-type Ca channels; $K_R = 146 \mu\text{M}$ and $K_I = 0.96 \mu\text{M}$ for block of the L-type Ca channels. 50 μM drug blocked both types of Ca channels almost completely.

Bepridil is also a potent blocker of T- and L-type Ca channels. Block of L-type Ca channels was steeply voltage dependent, but block of T-type Ca channels was not. Consequently, bepridil blocks T-type Ca channels more potently than L-type Ca channels when binding equilibrates at normal diastolic potentials (~ -90 mV). For T-type Ca channels, block by 0.5 μM bepridil indicates $K_I = 1.24 \mu\text{M}$ and block by 2 μM drug indicates $K_I = 1.38 \mu\text{M}$. Likewise, block of L-type Ca channels by 0.5 and 2 μM bepridil indicated $K_I = 0.20 \mu\text{M}$ and 0.16 μM , respectively.

The current recordings in the right column of Fig. 6 show substantial block of T-type Ca channels by 1 μM cinnarizine. Both components of Ca current were blocked by 5 μM cinnarizine, as shown in the two lower panels based on results from another cell. In this experiment, $K_I = 250$ nM for block of T-type Ca channels and

FIGURE 6. Block of L- and T-type Ca channels by amiodarone, bepridil, and cinnarizine. The results for each drug are shown in a separate column. For each drug, the top panel shows superimposed measurements of Ca channel currents made with and without the indicated concentration of drug and the middle and bottom panels show the effect of drug on the steady-state availability of T- and L-type Ca channels, respectively. Drug binding equilibrated at the indicated V_p for 30 s before each test pulse. The open squares indicate control measurements and the filled triangles indicate measurements in drug. Each set of data in the middle and bottom rows was fit by the equation $I/I_{\max} = [1 + \exp [(V_p - V_{1/2})/k]]^{-1}$. For amiodarone, the control availability for T-type Ca channels is defined by $I_{\max} = 115$ pA, $V_{1/2} = -70.6$ mV, and $k = 4.29$; in 10 μM drug, $I_{\max} = 96.4$ pA, $V_{1/2} = -81.0$ mV, and $k = 5.70$. For L-type Ca channels, $I_{\max} = 933$ pA, $V_{1/2} = -46.0$ mV, and $k = 4.15$ without drug; in 10 μM amiodarone, $I_{\max} = 873$ pA, $V_{1/2} = -56.0$ mV, and $k = 4.28$. For bepridil, the control curve for T-type Ca channels is defined by $I_{\max} = 537$ pA, $V_{1/2} = -68.0$ mV, and $k = 8.02$; in 0.5 μM bepridil, $I_{\max} = 429$ pA, $V_{1/2} = -68.9$ mV, and $k = 8.89$; in 2 μM drug (filled diamonds), $I_{\max} = 319$ pA, $V_{1/2} = -71.5$ mV, and $k = 10.72$. For L-type Ca channels, $I_{\max} = 1,618$ pA, $V_{1/2} = -33.4$ mV, and $k = 6.48$ without drug; in 0.5 μM bepridil, $I_{\max} = 1,532$ pA, $V_{1/2} = -42.0$ mV, and $k = 7.99$; in 2 μM bepridil (filled diamonds), $I_{\max} = 1,100$ pA, $V_{1/2} = -48.3$ mV, and $k = 7.04$. For cinnarizine, the control values for T-type Ca channels are $I_{\max} = 411$ pA, $V_{1/2} = -67.8$ mV, and $k = 4.90$; in 5 μM drug, $I_{\max} = 155$ pA, $V_{1/2} = -83.2$ mV, and $k = 10.0$. For L-type Ca channels, $I_{\max} = 1,002$ pA, $V_{1/2} = -32.2$ mV, and $k = 4.18$ without drug; in 5 μM cinnarizine, $I_{\max} = 478$ pA, $V_{1/2} = -51.4$ mV, and $k = 6.29$.

$K_i = 63$ nM for block of L-type Ca channels. Similar voltage-dependent block of T-type Ca channels by cinnarizine was seen in three other experiments, which contrasts with the lack of voltage dependence of block by bepridil seen in two experiments. In summary, all three drugs block T-type Ca channels more potently

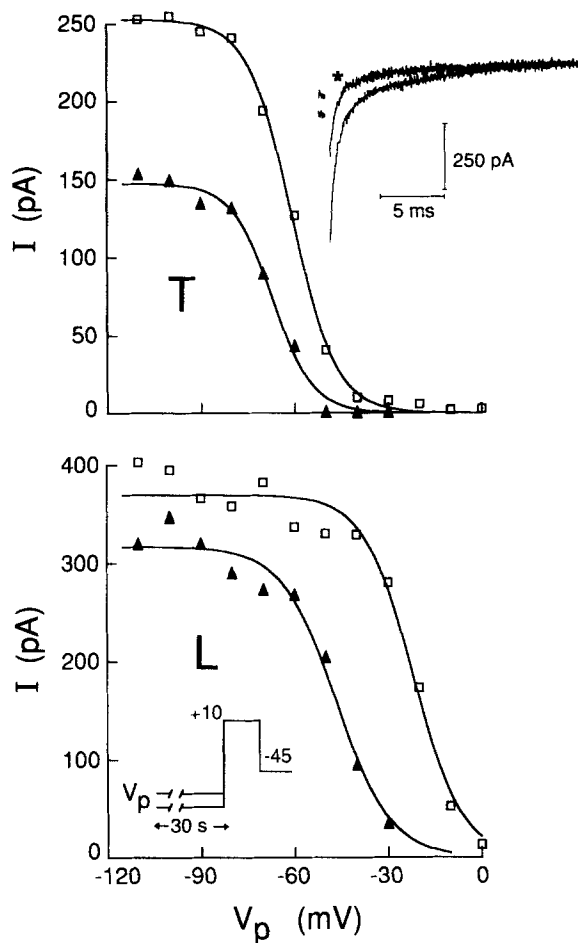


FIGURE 7. Block of L- and T-type Ca channels by felodipine. (*Top*) Steady-state availability of the T-type Ca channels measured with and without 15 nM felodipine. The amplitude of the slowly deactivating component of current is plotted vs. V_p . The membrane potential was held at V_p for 30 s before each test pulse. The open squares indicate control measurements and the filled triangles indicate measurements in drug. $I_{max} = 252.9$ pA, $V_{1/2} = -60.6$ mV, and $k = 7.05$ before drug and $I_{max} = 147.5$ pA, $V_{1/2} = -67.0$ mV, and $k = 6.51$ in felodipine. (*Inset*) Superimposed tail currents with and without 15 nM felodipine measured at -45 mV after a 10-ms test pulse to $+10$ mV from a holding potential of -70 mV. The record in drug is indicated by an asterisk. The control tail current was fit by two exponentials with time constants of 0.60 and 7.60 ms; in felodipine the time constants were 0.54 and 7.43 ms. (*Bottom*) The amplitude of the rapidly decaying component of tail current vs.

V_p . $I_{max} = 369.4$ pA, $V_{1/2} = -21.9$ mV, and $k = 7.79$ before drug and $I_{max} = 317.3$ pA, $V_{1/2} = -46.6$ mV, and $k = 8.69$ in felodipine. The inset shows the pulse protocol.

than L-type Ca channels when binding equilibrates at normal diastolic potentials, but high affinity binding to L-type Ca channels is more potent than to T-type Ca channels.

Ca channel block by quinidine was studied because this drug is useful for treating supraventricular tachycardia, which could be due to block of T-type Ca channels (Hagiwara et al., 1988). 50 μ M quinidine reduced the T-type Ca current by about

half with little or no dependence on holding potential. Quinidine block of T-type Ca channels is probably too weak to be relevant to the therapeutic action of this drug.

Fig. 7 shows the steady-state voltage dependence of block of L- and T-type Ca channels by the 1,4-dihydropyridine felodipine. The inset in the top panel shows superimposed measurements of Ca channel currents made with and without 15 nM felodipine when binding equilibrated at -70 mV. The current record in drug is indicated by an asterisk. Felodipine is a very potent blocker of the L-type Ca channels (as indicated by the block of the rapidly decaying component of tail current), but also causes substantial block of the T-type Ca channels (as indicated by the block of the slowly decaying component of tail current). The upper and lower panels of Fig. 7 show the effect of 15 nM felodipine on the steady-state availability of T- and L-type Ca channels, as in Fig. 6. Block of the T-type Ca channels is weakly voltage

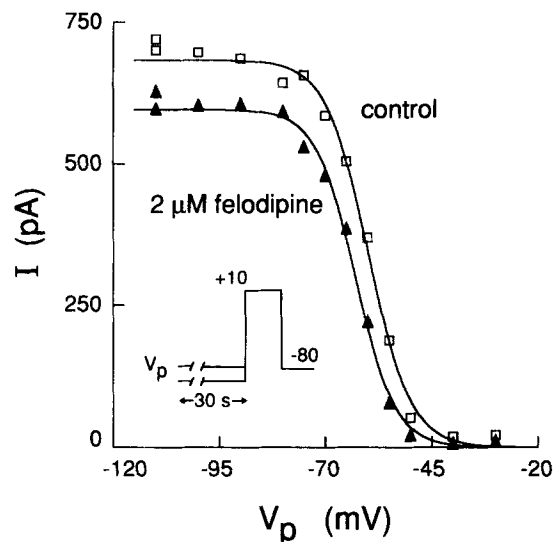


FIGURE 8. Felodipine block of the T-type Ca channels in GH₃ cells. The amplitude of the slow component of tail current is plotted as a function of V_p . The pulse protocol used for this experiment is shown in the left inset. The open squares indicate control measurements and the filled triangles indicate measurements in drug. The solid curve through each set of data points indicates the best fit by a two-state Boltzmann distribution. $I_{max} = 683$ pA, $V_{1/2} = -60$ mV, and $k = 5.0$ for the control data, and $I_{max} = 595$ pA, $V_{1/2} = -63$ mV, and $k = 4.6$ in $2 \mu\text{M}$ felodipine.

dependent, so that felodipine shifts the voltage dependence of T-type Ca channel availability to more negative potentials. Use of the modulated receptor theory indicated $K_I = 4.4$ nM in this experiment. The steady-state voltage dependence of block was also determined in the same cell with 5 nM felodipine and these data indicated $K_I = 7.4$ nM. Thus, the model accounts for both the voltage and concentration dependence of channel block. Similar results were obtained in five other experiments, with an average K_I of 12.8 ± 3.4 nM (\pm SEM). In one experiment, there was very little block with 25 nM felodipine and in another experiment there was little block by $1 \mu\text{M}$ drug.

As expected, felodipine is also a very potent blocker of L-type Ca channels in atrial cells. Quantitating this effect is difficult because felodipine can also increase L-type Ca channel currents when currents are elicited from well-polarized cells (data not

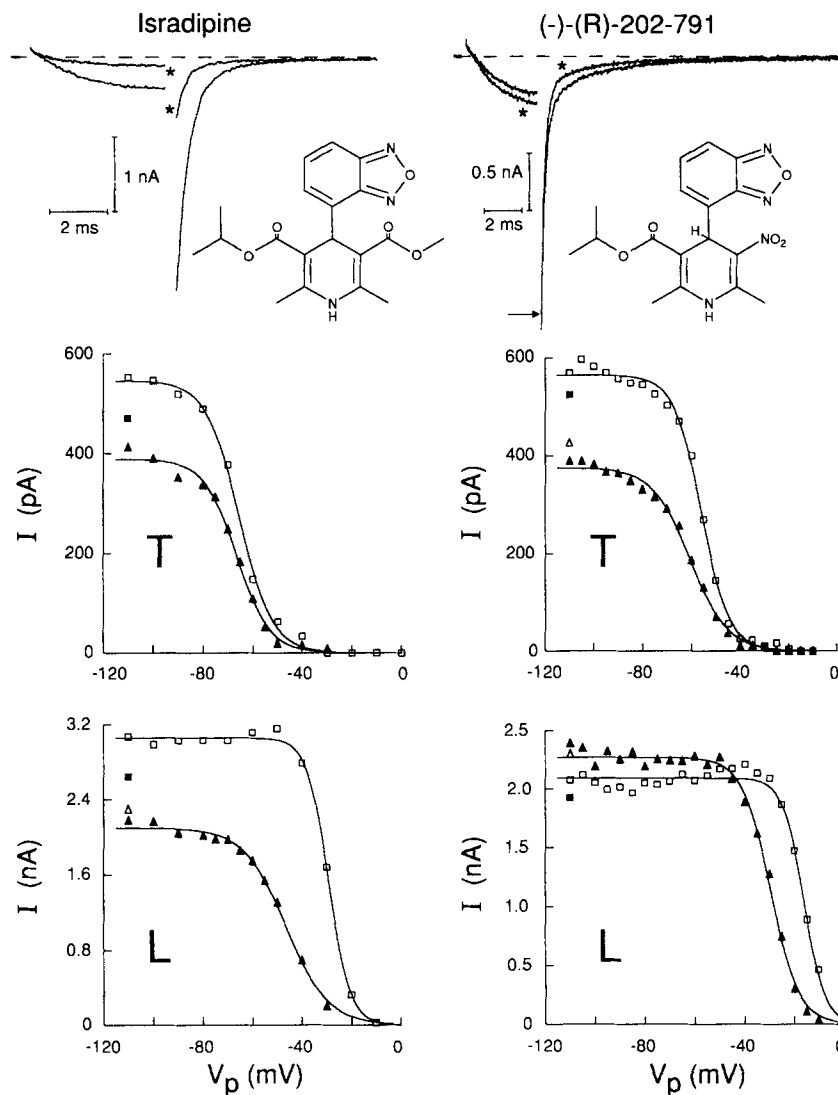


FIGURE 9. Block of Ca channels in guinea pig atrial cells by isradipine and (-)-(R)-202-791. The left-hand column shows the effect of 10 nM isradipine on L- and T-type Ca channel currents and the right-hand column shows the result of a different experiment with (-)-202-791. In the top panels, the records in drug are indicated by an asterisk. For each current record, a 30-s prepulse to -60 mV was followed by a 5-ms test pulse to $+30$ mV and the membrane was repolarized to -45 mV. The arrow in the top right panel indicates the amplitude of the control tail current measurement. The fast component of decay was fit with an exponential with $\tau = 0.35$ ms before drug and $\tau = 0.25$ ms in drug. If the increase in L-type current were due to contamination by the (+) enantiomer of drug, then the tail current would have decayed slower than control. The middle panels show plots of the amplitude of the slowly decaying component of tail current vs. V_p and the bottom panels show plots of the amplitude of the rapidly decaying component of tail current vs. V_p . The open squares indicate control measurements and the filled triangles indicate measurements in drug. To test for run-down, additional measurements

shown; similar increases in L-type Ca channel current have been seen with other dihydropyridine "Ca antagonists" [Brown, Kunze, and Yatani, 1986; Okabe, Kazumasa, Kitamura, and Kuriyama, 1987; Aaronson, Bolton, Lang, and MacKenzie, 1988] and can be seen in Fig. 9). It is not known whether the blocking and enhancing effects of felodipine are due to binding at a single site. The blocking effects of felodipine were observed in all experiments, but increases in Ca current were only observed in two of seven experiments, suggesting that two distinct mechanisms are involved. In experiments where only blocking effects were observed, an apparent K_1 of 0.43 ± 0.16 nM (\pm SEM) was calculated. Although the K_1 for felodipine block of the L-type Ca channels is much less than that for the T-type Ca channels, the effective binding constant for each channel type is not much different over a large portion of the physiologically relevant voltage range, as seen in the current records in the upper panel of Fig. 7. Inactivation of L-type Ca channels occurs at less negative potentials than for T-type Ca channels, so that most T-type Ca channels are in the high affinity state for $-70 < V < -50$ mV, while the L-type Ca channels are not.

The potent block of T-type Ca channels by felodipine is perhaps surprising because most previous studies with other 1,4-dihydropyridines have indicated relatively weak block of T-type Ca channels in a variety of cell types (Bean, 1989). We therefore evaluated the effects of felodipine on T-type Ca channels in GH₃ cells, which are derived from rat anterior pituitary cells and have previously been used to characterize Ca channel block by other 1,4-dihydropyridines (Cohen and McCarthy, 1987; Simasko, Weiland, and Oswald, 1988). The results of one of these experiments are shown in Fig. 8. The amplitude of the slow component of tail current is plotted vs. V_p . Felodipine is much less potent at blocking T-type Ca channels in GH₃ cells than in atrial cells. Use of the modulated receptor theory indicates that $K_1 = 1.7$ μ M. In six experiments, the average K_1 was 680 ± 220 nM (\pm SEM), so that block is ~ 50 -fold less potent than in atrial cells. Thus, substantial variations in the sensitivity of T-type Ca channels to block by dihydropyridines can occur between different cell types.

Fig. 9 shows that two other commonly used dihydropyridines also cause high

were made immediately before and after adding drug (*filled squares* and *open triangles*, respectively). For the middle left panel, $I_{\max} = 546.2$ pA, $V_{1/2} = -65.4$ mV, and $k = 6.67$ for the control data, and $I_{\max} = 388.4$ pA, $V_{1/2} = -66.4$ mV, and $k = 6.59$ in 10 nM drug. For the bottom left panel, $I_{\max} = 3.06$ nA, $V_{1/2} = -29.2$ mV, and $k = 4.31$ for the control data, and $I_{\max} = 2.09$ nA, $V_{1/2} = -46.4$ mV, and $k = 8.50$ in drug. These data indicate that $K_1 = 32$ nM for the T-type Ca channels and 0.63 nM for the L-type Ca channels. Steady-state Ca channel block was also determined with 25 nM drug in this experiment and the data indicated $K_1 = 14$ nM for the T-type Ca channels and 0.27 nM for the L-type Ca channels. For the middle right panel, $I_{\max} = 566$ pA, $V_{1/2} = -55.8$ mV, and $k = 5.68$ for the control data, and $I_{\max} = 376$ pA, $V_{1/2} = -60.5$ mV, and $k = 7.67$ in 25 nM drug. For the bottom right panel, $I_{\max} = 2.09$ nA, $V_{1/2} = -16.1$ mV, and $k = 4.25$ for the control data, and $I_{\max} = 2.27$ nA, $V_{1/2} = -29.6$ mV, and $k = 5.71$ in drug. These data indicate that $K_1 = 16.7$ nM for the T-type Ca channels and 1.78 nM for the L-type Ca channels (ignoring the increase in current at very negative V_p). Steady-state Ca channel block was also determined with 75 nM drug in this experiment and the data indicated $K_1 = 11.8$ nM for the T-type Ca channels and 1.50 nM for the L-type Ca channels.

affinity block of T-type Ca channels and that the structure–activity relationship for block of T-type Ca channels differs from that for block of L-type Ca channels. The left column of Fig. 9 shows the steady-state voltage dependence of Ca channel block by isradipine (PN 200-110) for a guinea pig atrial cell. Isradipine is a very potent blocker of L-type Ca channels often used in ^3H -ligand binding studies (Hof, Scholtysik, Loutzenhiser, Vuorela, and Neumann, 1984; Kokubun, Prod'hom, Becker, Porzig, and Reuter, 1986). The top left panel shows superimposed measurements of Ca channel currents made with and without 10 nM isradipine. Isradipine reduced the inward current during the test pulse and both components of tail current when the membrane was repolarized. The effects of (-)-202-791 under the same conditions are shown in the top right panel. This compound was tested because it is structurally similar to isradipine, but is known to be a weaker blocker of L-type Ca channels (Kokubun et al., 1986). Currents were increased by the drug during the test pulse, and the tail current measurements indicate that current through the L-type Ca channels was increased, while current through the T-type Ca channels was decreased. The four other panels of this figure show the steady-state voltage dependence of block of both types of Ca channels by both dihydropyridines. Channel block was assayed by tail current analysis, as described above. Isradipine and (-)-202-791 are approximately equipotent at blocking T-type Ca channels, with $K_1 = 20.4$ nM for isradipine and $K_1 = 15.3$ nM for (-)-202-791. However, these two compounds are quite dissimilar in their effect on L-type Ca channels, as is already apparent from the two top panels. As already indicated for our studies with felodipine, there is presently no theoretical framework that allows us to quantitate the two effects of (-)-202-791 on L-type Ca channels. If we ignore the increase in Ca current, then a K_1 of 4 nM is inferred, so that the ratio of the high affinity binding constants for the two types of Ca channels (T/L) is 3.6. The ratio of the high affinity binding constants for isradipine (T/L) is ~ 25 . This calculation correctly indicates that 1,4-dihydropyridines differ significantly in the relative affinity for the two types of Ca channels. However, the calculation is misleading in that it suggests that block of L-type Ca channels is more potent than block of T-type Ca channels for both drugs, when in fact (-)-202-791 selectively blocks T-type Ca channels at most voltages.

DISCUSSION

Our studies indicate that tail current analysis can be used to separate and quantitate current through L- and T-type Ca channels in guinea pig atrial cells. Tail current analysis allows us to study both types of Ca channels over a broad range of voltages and to evaluate block of both channel types simultaneously. This analysis revealed that: (a) block of T-type Ca channels by a number of commonly used Ca channel antagonists and antiarrhythmic agents is more potent than block of L-type Ca channels when binding equilibrates at normal diastolic potentials; and (b) some commonly used 1,4-dihydropyridines cause substantial block of T-type Ca channels at concentrations frequently used in pharmacological studies. Many pharmacological studies have attributed the effects of these same Ca channel antagonists to block of L-type Ca channels and may have thereby underestimated the role of T-type Ca channels in electrogenesis, excitation–contraction coupling, or stimulus–secretion coupling.

The Slowly Deactivating Component of Tail Current Provides a Reliable Indicator of the Instantaneous Conductance of T-Type Ca Channels

The two components of Ca channel tail current have different activation, inactivation, and deactivation kinetics and different selectivities for Ca vs. Ba. These results can best be explained by invoking two distinct populations of Ca channels similar to the L- and T-type Ca channels previously described in myocardial cells (Bean, 1985; Nilius et al., 1985; Mitra and Morad, 1986; Bonvallet, 1987; Hagiwara et al., 1988; Droogmans and Nilius, 1989; Hirano et al., 1989; Kawano and DeHaan, 1989; Tseng and Boyden, 1989; Tytgat et al., 1990; Xu and Best, 1990).

Our analysis of Ca channel block rests on the assertion that the slowly deactivating component of tail current is entirely due to current through T-type Ca channels. Changes in the amplitude of the tail current with changes in the concentration of bath Ca or Ba indicate that these divalent cations are the only significant charge carriers (see Figs. 3 and 4). The tail current was not through Na channels because it was unaffected by 30 μ M tetrodotoxin (data not shown). Slowly deactivating current through L-type Ca channels has been demonstrated in ventricular cells and was a particular concern for our studies (Bean and Rios, 1989; Pietrobon and Hess, 1990; Yue et al., 1990). Mode 2 activity is most prevalent after long test pulses to very positive potentials or during rapid trains of test pulses (Pietrobon and Hess, 1990). Slowly deactivating tail currents associated with L-type Ca channels could be detected in guinea pig atrial cells only after very strong test depolarizations (data not shown). Almost all of the tail current analysis evaluating block of T-type Ca channels was done using test pulse durations ≤ 10 ms and test potentials $\leq +30$ mV. The evidence against significant mode 2 activity under these conditions is as follows: (a) test pulses from a holding potential of -30 mV did not elicit slowly decaying tail currents (see Fig. 1); (b) the voltage dependence of inactivation of the slow component of tail current was the same for test potentials of $+10$ and $+60$ mV, even though activation of the L-type Ca channels is much greater at the more positive test potential (data not shown); (c) the slowly decaying component of tail current was not increased by substituting Ba for Ca as charge carrier, while currents through L-type channels were increased by this change (see Figs. 3 and 4); and (d) changes in the amplitude of the two components of tail current caused by Ca channel antagonists were not correlated (see Fig. 9).

Although most properties of the slowly deactivating Ca current are consistent with previous descriptions of T-type Ca current in myocardial cells, several significant differences exist. The Boltzmann distribution describing the voltage dependence of activation of slowly deactivating Ca current typically had a slope factor of ~ 15 (see Figs. 2, 3, and 5), while most studies of T-type Ca channels report a much steeper voltage dependence of activation, with slope factors ranging from 4.4 to 6.3 (Hagiwara et al., 1988; Hirano et al., 1989; Tytgat et al., 1990; Xu and Best, 1990). Some of the difference in activation kinetics may derive from methodological differences (we used an isochronal measurement of activation while previous studies calculated maximal conductance at each voltage), but genuine heterogeneity in the properties of T-type Ca channels apparently exists. Considerable variation in activation kinetics was found in our studies, with slope factors as low as 3 or as high as

23. It is interesting to note that slowly deactivating Ca channels in basilar artery smooth muscle have a shallow voltage dependence of activation and are highly sensitive to block by nifedipine (Simard, 1991).

Our results indicate methods for enhancing the isolation of T-type Ca currents. The separation of Ca channel currents into low voltage-activated and high voltage-activated components is enhanced by using Ca rather than Ba as the charge carrier and by increasing the bath pH (see Figs. 3 and 5). Many studies of T-type Ca channels use Ba as charge carrier in order to prevent Ca-induced inactivation of L-type Ca channels and thereby better separate the two components of current based on their rate of inactivation. However, this advantage is negated by buffering cell Ca with high concentrations of BAPTA in the pipette solution. Ba is also sometimes used as charge carrier to maximize the amplitude of L-type Ca currents, but we found that L-type currents are not always increased when Ba is substituted for Ca as the charge carrier (see Fig. 4).

Block of T-Type Ca Channels by Ca Channel Antagonists and Antiarrhythmic Agents

L-type Ca channels have many distinct binding sites for Ca antagonists (Janis, Silver, and Triggle, 1987; Hosey and Lazdunski, 1988). The three best-described binding sites are those for 1,4-dihydropyridines, verapamil, and diltiazem, but many structurally dissimilar classes of compounds can block L-type Ca channels. The potent block of T-type Ca channels by amiodarone, bepridil, cinnarizine, and felodipine indicates that T-type Ca channels have multiple binding sites for drugs similar to those on L-type Ca channels.

Cinnarizine binds to a site on L-type Ca channels distinct from that for dihydropyridines, diltiazem, or verapamil (Siegl, Garcia, King, Scott, Morgan, and Kaczorowski, 1988). Cinnarizine block of T-type Ca channels was particularly interesting for two reasons: (a) the high affinity binding constant (K_i) of cinnarizine for T-type Ca channels is comparable to that for L-type Ca channels, while all other drugs studied bind more strongly to L-type Ca channels; and (b) block of T-type Ca channels is strongly voltage dependent, in a manner similar to the block of Na channels and L-type Ca channels by therapeutically useful drugs. Voltage-dependent channel block can account in part for the tissue specificity of class I antiarrhythmic agents and clinically used Ca antagonists (Hondegheem and Katzung, 1984). Flunarizine, a congener of cinnarizine, blocks T-type Ca channels in aortic smooth muscle, ventricular myocytes, and hippocampal and hypothalamic neurons (Tytgat et al., 1988; Akaike et al., 1989a; Akaike, Kostyuk, and Osipchuk, 1989b; Kuga et al., 1990; Takahashi and Akaike, 1991). The potency of flunarizine block of T-type Ca channels differs dramatically in these studies. Hence, studies with flunarizine support our conclusion, based on studies with felodipine, that T-type Ca channels are pharmacologically heterogeneous.

Bepridil is an arylalkylamine analogue of verapamil. Inhibition of verapamil binding to L-type Ca channels is consistent with competitive block (Garcia, Trumble, Reuben, and Kaczorowski, 1984; Galizzi, Borsotto, Barhanin, Fosset, and Lazdunski, 1986). Although verapamil and gallopamil are reportedly weak blockers of T-type Ca channels in myocardial cells (Hagiwara et al., 1988; Tytgat et al., 1988), we found fairly potent block by bepridil. Bepridil block of T-type Ca channels is more potent than block of Na channels or L-type Ca channels when binding equilibrates in

well-polarized cells (see Fig. 8 and Yatani et al., 1986), which may account in part for the activity of this drug in treating supraventricular arrhythmias. Block of L-type Ca channels in guinea pig ventricular cells similar to our results has been reported (Yatani et al., 1986).

Amiodarone is effective in treating some forms of ventricular arrhythmias that are resistant to other drugs (Mason, 1987). The mechanism of action of this drug is ill-defined because many ion channels are blocked by drug. The most potent block is of Na channels and L-type Ca channels (Follmer, Aomine, Yeh, and Singer, 1987; Nishimura et al., 1989). The apparent binding constants for the inactivated state of Na and L-type Ca channels are 120 and 360 nM, respectively. It is difficult to compare our results with amiodarone with previous studies because the onset of channel block is very slow. However, block of T-type Ca channels is likely to be relevant to the therapeutic activity because block of T-type Ca channels is approximately equipotent to that for L-type Ca channels (see Fig. 8, with $K_1 \approx 1 \mu\text{M}$ for both channel types).

Although amiodarone, bepridil, and cinnarizine block T-type Ca channels more potently than L-type Ca channels when binding equilibrates at normal diastolic potentials, the relative potency of Ca channel block may differ in working myocardium that is electrically driven.

Our results with felodipine and isradipine are not inconsistent with previous reports that some T-type Ca channels are relatively insensitive to block by 1,4-dihydropyridines. High affinity block of T-type Ca channels by felodipine is tissue specific (see Figs. 7 and 8). Block of L-type Ca channels by dihydropyridines also differs substantially between different cell types and the partition coefficient of these drugs into the membrane may be an important factor in accounting for the tissue specificity of block (McCarthy and Cohen, 1989). Similar factors may contribute to the tissue specificity of block of T-type Ca channels. Most previous studies also used dihydropyridines other than those reported on in this study and the choice of drug is significant. For example, nifedipine and nimodipine are significantly weaker blockers of T-type Ca channels in guinea pig atrial cells than felodipine or isradipine, with $K_1 \approx 100 \text{ nM}$ (data not shown). Finally, most previous studies with dihydropyridines did not evaluate block of the L- and T-type Ca channels at the same voltage; block of L-type Ca channels is often shown only in depolarized cells, where block is most potent.

High affinity block of T-type Ca channels by 1,4-dihydropyridines has previously been reported in aortic and portal vein smooth muscle cells (Loirand, Mironneau, Mironneau, and Pacaud, 1989; Kuga et al., 1990). In these studies, T-type Ca channel current was isolated by dialyzing the cell with solutions that contained F^- or high concentrations of Ca ions. Hence, the potency of block of L- and T-type Ca channels was evaluated in parallel experiments under different ionic conditions. Our studies allowed simultaneous evaluation of block of both channel types, with qualitatively similar results. However, we never observed increases in T-type Ca current produced by dihydropyridines, such as the nifedipine-induced increase observed in aortic smooth muscle (Kuga et al., 1990).

Several other previous studies suggest potent block of T-type Ca channels by dihydropyridines: (a) Smooth muscle cells from rabbit mesenteric arteries contain a Ca channel with a conductance of 10 pS that is blocked by nisoldipine with high

affinity (Worley, Deitmer, and Nelson, 1986). L-type Ca channels usually have a conductance of ~ 25 pS, while T-type Ca channels have a conductance of ~ 9 pS (Bean, 1989). (b) All of the Ca current in smooth muscle cells from rabbit ear artery is potently blocked by nifedipine (Aaronson et al., 1988) and these cells contain both L- and T-type Ca channels (Benham, Hess, and Tsien, 1987). (c) Noradrenaline increases T-type Ca channel current and decreases L-type Ca channel current in rat portal vein cells and the contractions induced by noradrenaline are potently blocked by dihydropyridines (Dacquet, Mironneau, and Mironneau, 1987; Pacaud, Loirand, Mironneau, and Mironneau, 1987). (d) Nitrendipine blocks T-type Ca channels in adrenal glomerulosa cells, with $K_1 = 190$ nM (Cohen et al., 1988). Nitrendipine is probably a weaker blocker of T-type Ca channels than felodipine or isradipine.

The T-type Ca channels in atrial cells have many properties in common with the dihydropyridine-sensitive Ca channels in skeletal muscle. Both channel types have a primary conductance of ~ 10 pS, are less sensitive to dihydropyridine block than cardiac L-type Ca channels, show little or no Ca-dependent inactivation, and have similar conductances for Ca and Ba (Rosenberg, Hess, Reeves, Smilowitz, and Tsien, 1986; Smith, McKenna, Ma, Vilven, Vaghy, Schwartz, and Coronado, 1987; Beam and Knudson, 1988). A major difference between the skeletal muscle channel and cardiac T-type Ca channels is that current is enhanced by BAY K 8644 only for the skeletal muscle channel. However, the same gene codes for both the skeletal muscle channel and dihydropyridine-sensitive charge movement, and charge movement is not increased by BAY K 8644 (Lamb and Walsh, 1987; Rios and Brum, 1987; Tanabe, Beam, Powell, and Numa, 1988).

1,4-Dihydropyridines have often been used as probes to test for the involvement of L-type Ca channels in Ca-dependent signal transduction. Drug effects are often evaluated in cells that are relatively well polarized or are only depolarized for brief periods during trains of action potentials. Our results indicate that dihydropyridines may not be selective blockers of L-type Ca channels if block is assayed under conditions where T-type Ca channels are available to open. A similar conclusion was reached in studies with rat aortic smooth muscle and hypothalamic neurons (Akaike et al., 1989a, b; Kuga et al., 1990). The potency of dihydropyridine block of L-type Ca channels seems to vary substantially between different tissues, with much weaker block in some neuronal and endocrine cells than in myocardial or smooth muscle cells (Boll and Lux, 1985; Gahwiler and Brown, 1987; Holz, Dunlap, and Kream, 1988; but see Jones and Jacobs, 1990). Hence, the block of T-type Ca channels in guinea pig atrial cells is much more potent than block of L-type Ca channels in some other tissues. Consequently, the role of T-type Ca channels in excitation-contraction coupling or stimulus-secretion coupling may have been greatly underestimated.

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