Decoding of synaptic voltage waveforms by specific classes of recombinant high-threshold Ca\textsuperscript{2+} channels

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Studies suggest that the preferential role of L-type voltage-sensitive Ca\textsuperscript{2+} channels (VSCCs) in coupling strong synaptic stimulation to transcription is due to their selective activation of local chemical events. However, it is possible that selective activation of the L-type channel by specific voltage waveforms also makes a contribution. To address this issue we have examined the response of specific Ca\textsuperscript{2+} channel types to simulated complex voltage waveforms resembling those encountered during synaptic plasticity (gamma and theta firing frequency). L-, P/Q- and N-type VSCCs (α\textsubscript{1C}, α\textsubscript{1A}, α\textsubscript{1B}/β\textsubscript{1A}/α\textsubscript{2B}, respectively) were all similarly activated by brief action potential (AP) waveforms or sustained step depolarization. When complex waveforms containing large excitatory postsynaptic potentials (EPSPs), APs and spike accommodation were applied under voltage clamp we found that the integrated L-type VSCC current was approximately three times larger than that produced by the P/Q- or N-type Ca\textsuperscript{2+} channels (gamma frequency 1 s stimulation). For P/Q- or N-type channels the complex waveforms led to a smaller current than that expected from the response to a simple 1 s step depolarization to 0 or +20 mV. EPSPs present in the waveforms favoured the inactivation of P/Q- and N-type channels. In contrast, activation of the L-type channel was dependent on both EPSP- and AP-mediated depolarization. Expression of P/Q-type channels with reduced voltage-dependent inactivation (α\textsubscript{1A}/β\textsubscript{2A}/α\textsubscript{2B}) or the use of hyperpolarized intervals between AP stimuli greatly increased their response to complex voltage stimuli. We propose that in response to complex synaptic voltage waveforms P/Q- and N-type channels can undergo selective voltage-dependent inactivation leading to a Ca\textsuperscript{2+} current mediated predominantly by L-type channels.

(Received 14 July 2003; accepted after revision 12 September 2003; first published online 18 September 2003)

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A combination of molecular biological and electrophysiological characterization studies has revealed a significant degree of structural and functional heterogeneity for the different voltage-sensitive Ca\textsuperscript{2+} channel (VSCC) types (Zhang \textit{et al.} 1993; Ertel \textit{et al.} 2000). Broadly, VSCCs fall into two categories based on their physiological and pharmacological profiles (Zhang \textit{et al.} 1993). The first category includes VSCCs that require high levels of depolarization for their activation and includes the L-, P/Q-, N- and R-types. The second category is composed solely by T-type VSCCs and requires lower levels of depolarization for activation. More recent nomenclature gives rise to three structurally and functionally related families (Ca\textsubscript{1}, Ca\textsubscript{2} and Ca\textsubscript{3}) (Ertel \textit{et al.} 2000). The L-, P/Q- and N-type channels in our study correspond to Ca\textsubscript{1.2}, Ca\textsubscript{2.1} and Ca\textsubscript{2.2} respectively. R-type currents can be attributed in part to α\textsubscript{1E} subunit (Ca\textsubscript{2.3}). The low-threshold activated T-type channel corresponds to the Ca\textsubscript{3} family and consists of three channel types, Ca\textsubscript{3.1}, Ca\textsubscript{3.2} and Ca\textsubscript{3.3}. The subunit composition of the different VSCCs also varies and has dramatic effects on channel inactivation (Stotz & Zamponi, 2001). L-, P/Q- and N-type channels display varying degrees of Ca\textsuperscript{2+} and voltage-dependent inactivation, whereas the L-type channel undergoes predominantly Ca\textsuperscript{2+}-dependent inactivation (Yue \textit{et al.} 1990; Randall & Tsien, 1995). Experimental evidence suggests a selective role for L-type VSCCs in activating immediate early gene expression in response to stimuli that mimic robust synaptic stimulation (Murphy \textit{et al.} 1991; Bading \textit{et al.} 1993; Dolmetsch \textit{et al.} 2001; Weick \textit{et al.} 2003). The preferential role of the L-type channel is thought to result from its selective coupling to intracellular signal transduction pathways triggered by specific spatial and temporal properties of Ca\textsuperscript{2+} signalling (Murphy \textit{et al.} 1991; Bading \textit{et al.} 1993; Dolmetsch \textit{et al.} 2001; Weick \textit{et al.} 2003). However, it is also possible that selective activation of the L-type channel in response to particular voltage stimuli in combination with local Ca\textsuperscript{2+}-dependent chemical events provides an even more selective mechanism for stimulus–transcription coupling. The biophysical properties of the L-type VSCC, including its...
relatively slow inactivation (Zhang et al. 1993), suggest that it is an ideal sensor for long-lasting bursts of activity that lead to steady depolarization (Nakazawa & Murphy, 1999; Meremelstein et al. 2000). We have extended these previous studies by performing voltage-clamp experiments on recombinant L-, P/Q- and N-type VSCCs expressed in human embryonic kidney (HEK) 293 cells using complex waveforms that mimic synaptic stimuli at physiological temperature as voltage commands.

Our experiments involve first producing plausible EPSP and AP waveforms using a computer simulation of CA1 hippocampal neuron synaptic physiology and then testing whether specific VSCC subtypes are tuned to these physiologically relevant waveforms (i.e. gamma and theta firing frequencies) (Christie et al. 1995a; Thomas et al. 1998). Surprisingly, our initial experiments at physiological temperature and with Ca\(^{2+}\)-dependent inactivation present indicate little difference in the response of L-, P/Q- and N-type channel types to prolonged step depolarization (1 s). Therefore, we set out to determine whether differential activation of VSCC subtypes might occur under conditions of physiological temperature and divalent ion concentration in response to complex voltage waveforms that more closely mimic synaptic stimuli. Under these conditions we observed that complex synaptic burst waveforms led to a selective voltage-dependent inactivation of non-L-type VSCCs that was not readily predicted by the response of the channels to simple prolonged step depolarization. This selective inactivation process leads to a Ca\(^{2+}\) current with a significantly larger L-type component. We suggest that the tuning of L-type channels to specific voltage waveforms, together with their preferential link to intracellular signal transduction, collaborate to give the channels a unique role in coupling synaptic stimulation to transcriptional activation (Murphy et al. 1991; Bading et al. 1993; Dolmetsch et al. 2001).

### METHODS

**Hippocampal CA1 neuron model**

We performed our simulation with the program NEURON (version 4.1.1) (Hines & Carnevale, 1997) on a PII-450 PC, using a 25 μs time step. The purpose of the model was to create plausible somatic waveforms for pyramidal neuron activity during high-frequency stimulation. Simulated recording have inherent advantages including the ability to easily alter stimulation frequency or particular conductance.

Studies by Mainen & Sejnowski (1998) indicate that the complex spiking behaviour of pyramidal neurons can be modelled using relatively few compartments. For our CA1 neuron model we have used four compartments that include a central soma as well as two dendritic processes and an axon. The model cell has the following passive membrane parameters: axial resistance (\(R_a\)) = 100 Ω cm (for dendrites and soma, 50 Ω cm for axon), membrane resistance (\(R_m\)) = 50 000 Ω cm\(^2\), membrane capacitance (\(C_m\)) = 1 μF cm\(^{-2}\). Conductances were placed in these various compartments at levels indicated based on experimental data (see Table 3, Appendix). To simplify the model, synaptic conductances were placed along the dendrites at 100, 300 and 700 μm positions. The axon hillock was defined as a short segment between the axon and the soma. To simulate a lower threshold for AP initiation within the axon hillock region we have added a higher concentration of voltage-gated Na\(^{+}\) conductance. Although this results in back-propagating APs, it is possible that in actual neurons there are alternative mechanisms (Colbert & Pan, 2002).

A notable feature of hippocampal CA1 pyramidal neurons is the presence of back-propagating APs (Spruston et al. 1995; Stuart et al. 1997b). Recent data suggest that a gradient (highest in distal regions) of dendritic A-type K\(^{+}\) current (\(I_A\)) also plays an important role in attenuating dendritic back-propagating APs in hippocampal CA1 neurons (Hoffman et al. 1997). Accordingly, we have included a gradient of \(I_A\) expression as in Hoffman et al. (1997). To study the activation of VSCCs during stimuli that are relevant to synaptic plasticity, we have modelled the effect of 100 Hz trains of presynaptic stimulation on postsynaptic membrane potential in the soma. Our experimental data suggest that hippocampal CA1 neurons exhibit a complex firing behaviour in response to 100 Hz tetanic stimulation (data not shown). Notable features of the response to tetanic stimulation include spike accommodation as well as voltage-dependent inactivation of Na\(^{+}\) currents (Fleidervish et al. 1996; Mickus et al. 1999). These two phenomena lead to a slowing of AP frequency and a reduction in amplitude. It has been reported that in hippocampal CA1 neurons, one type of voltage-activated K\(^{+}\) channel (\(I_{\text{SK}}\)) and three types of Ca\(^{2+}\)-activated K\(^{+}\) channels (BK, \(s_{I_{\text{AHP}}}\) and SK) are responsible for the spike frequency accommodation (Sah & Bekkers, 1996; Halliwell & Adams, 1982; Shao et al. 1999). The properties of these K\(^{+}\) channels were modelled according to reported experimental data. To produce voltage-dependent inactivation of Na\(^{+}\) channels we added a slow variable, \(s\), into the classical Hodgkin–Huxley-type Na\(^{+}\) channel model and were able to obtain a frequency- and voltage-dependent inactivation of Na\(^{+}\) channels during AP trains (Mickus et al. 1999). Comparison of simulated 100 Hz synaptic stimulus trains (Fig. 2) to experimental data from hippocampal pyramidal neurons (Egorov et al. 1999; Yeckel et al. 1999) indicates good qualitative correspondence. One of the most critical parameters for generation of different firing patterns is the level of synaptic conductance, which represents the combined open channel conductance of all NMDA and AMPA receptors. The synaptic conductance of the model was set to 0.0075 μS to generate a single AP and was increased to 0.01 and 0.3 μS to produce the repetitive and plateau waveforms respectively (100 Hz presynaptic stimulation). Theta stimulation was produced by delivering presynaptic burst stimulation with a 7.5 Hz frequency and either a strong or a weak synaptic conductance (0.01 and 0.3 μS). GABAergic mechanisms were not included in the model (see Appendix for more detailed mechanisms).

**Preparation of HEK 293 cells**

HEK 293 cells (CRL 1573; ATCC, Rockville, MD, USA) were passaged once every 2–4 days. For calcium phosphate transfection cells were plated at a density of 1 x 10\(^6\) cells ml\(^{-1}\) in 10 cm culture dishes (Falcon, Becton Dickinson Labware, Franklin Lakes, NJ, USA). Cells were transfected for 6–8 h with cDNAs encoding the rat brain \(\alpha_1\) (\(\alpha_{1A}\) or \(\alpha_{1C}\)), \(\alpha_2\) and \(\beta\) subunit (\(\beta_{18}\) or \(\beta_{3}\)) (obtained from Dr T. Snutch, University of British Columbia, Canada) at a ratio of 1:1:1 with a total of 12 μg plasmid cDNA for a 10 cm culture plate. After a period of 16–24 h following transfection,
cells were transferred to a 29°C incubator (in some experiments 37°C) for up to 5 days (Stotz & Zamponi, 2001). Recordings were initiated 36 h after the termination of transfection. For the N-type VSCC, the HEK ts201 cell line stably expressing α1B, α2 and β1B subunits (obtained from Dr Snutch) was used. The N-type channel α subunit (rB-II isomorf) was in the unmodulated G177 form, which features the ‘willing’ state with fast activation and susceptibility to G-protein inhibition (Zhong et al. 2001). Our α1A construct represents a splice variant of α1A, subtype, which is missing both a valine insertion (Val421) in I–II linker as in P-like channels and the insertion of asparagine plus proline (N1605–P1606) into the S3–S4 extracellular linker of repeat IV as in Q-like channels (Bourinet et al. 1999). This α1A subtype may correspond to a novel P-like channel in the cerebellum with a functional profile intermediate between P- and Q-like channels (Tontone et al. 1996).

**Electrophysiology**

Voltage-clamp recording was performed at both room temperature (21.5–23°C) and physiological temperature (35–37.5°C) on the stage of an inverted microscope (Aiviovert, Zeiss). Electrodes with open tip resistances of 1–3 MΩ were used. The intracellular recording solution for the HEK 293 cells contained (m M): 120 CsMeSO 3, 5 CsCl, 10 EGTA, 1 m be less than 50 in Q-like channels (Bourinet et al. 1999). This α1A subtype may correspond to a novel P-like channel in the cerebellum with a functional profile intermediate between P- and Q-like channels (Tontone et al. 1996).

**RESULTS**

L-, P/Q- and N-type voltage sensitive Ca 2+ channels all respond to brief action potential-like stimuli

Using simulated AP and EPSP waveforms as voltage commands (McCobb & Beam, 1991; Patil et al. 1998), we have conducted whole-cell voltage-clamp experiments on HEK 293 cells expressing different recombinant VSCCs. We have contrasted the activation of the α1A (P/Q-type), α1B (N-type) and α1C (L-type) subunits expressed in combination with the β1B (or in some cases β2A) and α2 subunits. To perform our experiments under more physiological conditions we elevated the bath temperature to ~37°C (range 35–37.5°C) and observed a marked increase in the activation rate as well as a speeding of inactivation of all channel types (see Fig. 1A and B, Tables 1 and 2). In these experiments we used 2 mM Ca 2+ as the charge carrier for characterization of VSCCs and the expression of the β1B subunit to preserve voltage and Ca 2+ -dependent inactivation expected under physiological conditions in neurons (Parnell et al. 1991; Ludwig et al. 1997). The peak current was also significantly increased (>300%) compared to responses recorded at room temperature (22°C) for the three channel types studied (Table 1). Surprisingly, we found that the three channel types at 37°C produced qualitatively similar responses to the strong step depolarization (Fig. 1A and B). At 37°C less than a 40% difference in the integrated Ca 2+ current over a 1 s period was observed when L-, P/Q- and N-type channels were compared (data normalized to peak response, see Methods). Elevated temperature was also associated with a hyperpolarizing shift in the voltage at which half-maximal channel activation occurred for L- and P/Q-type channels (V 0.5 = 22/37°C; L: −10.2 ± 3.1/−20.0 ± 1.3 mV; P/Q: −12.6 ± 0.9/−23.2 ± 1.2 mV; and N: 6.2 ± 1.5/5.5 ± 2.6 mV; see Fig. 1C for 37°C data). The conductances were fit to the Boltzmann equation (Tateyama et al. 2001) and the V 0 was determined. The reversal potentials used for calculating the conductance were 50.7 ± 1.8 mV (n = 12), 49.4 ± 1.8 mV (n = 14) and 53.1 ± 2.4 mV (n = 12) for L-, P/Q- and N-type channels respectively. L-type VSCCs were markedly more resistant to steady-state inactivation than the P/Q- or N-type; 50% channel inactivation at ~64 mV for both P/Q- and N-type versus ~35 mV for L-type VSCCs (Fig. 1D). The voltage dependence of inactivation was similar to published values for P/Q- and N-type channels (Patil et al. 1998; Sutton et al. 1999). In this study, we have termed the inactivation state at the end of a 1 s step depolarization ‘steady-state inactivation’, although it is possible that a more slowly developing component (<10% amplitude) may also be involved.

Previous studies have suggested that L-type VSCCs respond preferentially to a large long-lasting depolarization and do not respond as well to brief stimuli such as single APs (Murphy et al. 1991; Mermelstein et al. 2000).
Figure 1. Activation and inactivation of VSCCs in response to step depolarization

A, at room temperature (~22°C) L-, P/Q- and N-type channels expressed in HEK 293 cells have relatively slow activation and inactivation kinetics (1 s step depolarization from -70 to 0 mV, except +20 mV for N-type). B, at 37°C all three channel types studied exhibited dramatically faster activation and inactivation. Overplotted normalized records (to peak current) of L-, P/Q- and N-type VSCC currents in response to the 1 s step depolarization are shown in A and B. Group data indicate that the normalized integrated L-type VSCC current (integral in pC divided by peak current; pC pApeak⁻¹) is only moderately larger than the other two channel types in response to the 1 s depolarization (significant difference between the L- and N-type channels; P < 0.01 one-way ANOVA). For this analysis the currents were integrated over the 1 s waveform and normalized to the peak current (n = 17, 21 and 14 for L-, P/Q- and N-type respectively).

C, voltage dependence of L-, P/Q- and N-type channel activation at 37°C determined by monitoring the peak current response at the indicated potentials and expressing the data as a conductance change (g gmax⁻¹). The conductances were fit to the Boltzmann equation and the Vₐ was determined.

D, channel inactivation determined by a 10 ms test pulse from -100 to 0 mV given 2–5 ms after a 1 s inactivating step to 0 mV from the indicated holding potentials. The peak currents elicited at 0 mV were then normalized to the maximal response obtained and fitted to the Boltzmann equation to determine the voltage at which 50% of the channels were inactivated: P/Q- (~64 mV, n = 5) and N-type Ca²⁺ channels (~64 mV, n = 8) have a markedly more negative inactivation profile than L-type channels (~35 mV, n = 7).

E, step pulses of limiting duration indicate that APs effectively activate L-, P/Q- and N-type channels. A series of short step depolarizing pulses from -70 to +25 mV of varied duration from 0.1 to 3.1 ms were given to mimic APs of varying duration. An example of an overplotted L-type VSCC response to the short step pulses at 22 and 37°C.
However, these studies were limited in that they were performed on a heterogeneous population of channels at room temperature. The studies also failed to describe how L-type VSCCs are activated by bursts of APs and EPSPs associated with synaptic plasticity (Christie et al. 1995b; Thomas et al. 1998). Under more physiological conditions, neurons would rarely experience steady depolarization in the form of EPSPs to potentials more positive than −40 mV (Storm & Hvalby, 1985). To further address this issue, voltage steps (to +25 mV) of different lengths were used to simulate APs of limiting duration (Fig. 1E). At 22°C we observed that step widths of <1 ms resulted in <50% of maximal channel activation for L-, P/Q- and N-type channels. However, at 37°C we observed that all three channel types were effectively activated (up to 90%) by AP mimicking steps of ~1.0 ms (Fig. 1E and F). Using briefer pulses (<0.5 ms) we observed that the P/Q-type channel was more effectively activated than the N- or L-type channels (P < 0.05), consistent with the shorter rise time of the P/Q-type channel at 37°C shown in Table 1.

### Table 1. Characteristics of L-, P/Q- and N-type VSCCs derived from 1 s step depolarization

<table>
<thead>
<tr>
<th>Channel Type</th>
<th>Rise time (ms)</th>
<th>Peak (pA)</th>
<th>Residual ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>22°C</td>
<td>37°C</td>
<td>22°C</td>
</tr>
<tr>
<td>L</td>
<td>β₀₀</td>
<td>3.2 ± 0.2</td>
<td>0.6 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>18</td>
<td>n</td>
</tr>
<tr>
<td>P/Q</td>
<td>β₀₀</td>
<td>13.4 ± 0.9</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>9</td>
<td>n</td>
</tr>
<tr>
<td>N</td>
<td>β₀₀</td>
<td>2.3 ± 0.7</td>
<td>0.5 ± 0.04</td>
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<tr>
<td></td>
<td>n</td>
<td>4</td>
<td>n</td>
</tr>
<tr>
<td></td>
<td>β₀₀</td>
<td>1.1</td>
<td>—</td>
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<tr>
<td></td>
<td>n</td>
<td>1</td>
<td>n</td>
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</table>

VSCC, voltage-sensitive Ca²⁺ channel. The rise times between 22 and 37°C were significantly different for all channel types studied (P < 0.001). The residual ratio is the percentage of steady-state current value at the end of 1 s depolarization over the step peak current. α₁₃ (P/Q-type), α₂₃ (N-type), and α₃₄ (L-type) subunits were expressed in HEK 293 cells in combination with β₁₀ and β₁₅ subunits. Data shown are the means ± S.E.M. of data obtained from HEK 293 cells expressing L- (n = 8/22, 22/37°C), P/Q- (n = 8/9) or N-type channels (n = 8/12).

Selective response of L-type voltage-sensitive Ca²⁺ channels to simulated gamma and theta frequency synaptic activity

In contrast to the effect of step depolarization, application of more complex and physiologically relevant simulated waveforms, corresponding to 1 s of gamma and theta presynaptic stimuli, led to marked differences in VSCC kinetics and in integrated current (over 1 s) when the three channel types were compared (Fig. 2A and B). In simulating the postsynaptic (somatic) response of a CA1 hippocampal neuron to high-frequency presynaptic stimulation we included mechanisms responsible for many of the salient features of postsynaptic activity including spike accommodation, EPSPs and Na⁺ channel inactivation. To control for differences in absolute expression levels we normalized the area of the Ca²⁺ current for the test waveforms to the peak amplitude of a preceding step response in which maximal channel activation occurred (see Methods). The normalized integrated Ca²⁺ current through the L-type VSCC in response to a complex waveform containing both EPSPs and APs (termed the repetitive firing waveform, reflects postsynaptic EPSPs and APs stimulated by a 100 Hz presynaptic tetanus) was considerably larger than that mediated by the P/Q- or N-type VSCC (335 and 518% of the P/Q- and N-type responses respectively; see Fig. 2A and B). To stimulate more robust levels of depolarization we also simulated a plateau-type waveform by increasing the simulated synaptic conductance (30-fold). In this case the simulated neuron was depolarized to a steady level of −33 mV and again the integrated L-type response was considerably better than the P/Q-type or the N-type (288%, and 679% of the P/Q- and N-type responses respectively; see Fig. 2A and B). In response to the plateau much of the current entry through the L-type VSCC as well as the difference in total Ca²⁺ influx between channel types was associated with the plateau itself and appeared as a...
Figure 2. Ca\textsuperscript{2+} currents in response to simulated waveforms

A, the upper panel shows 1 s duration computer-simulated voltage waveforms used as command potentials to activate the VSCCs (delivered from \textasciitilde\textasciitilde70 mV). The lower three panels show the Ca\textsuperscript{2+} current at 37°C in response to the corresponding voltage waveform above them. B, group data for the results shown in A. Each column was obtained by integrating the total Ca\textsuperscript{2+} current in response to the corresponding voltage waveform (units pC) and subsequently normalizing this to the peak current response (units nA) of a preceding step depolarization (see Methods; expressed as pC nA\textsubscript{peak}\textsuperscript{-1}). The results indicate a considerably larger integrated Ca\textsuperscript{2+} current mediated by the L-type channel in response to complex voltage waveforms. L-type channel activation in response to the repetitive, plateau, and strong theta waveforms resulted in a significantly larger normalized Ca\textsuperscript{2+} integrated current than that observed from P/Q- or N-type channels (\textit{P} < 0.001; \textit{n} > 10 for all groups). In the case of the weak theta waveform only a modest difference was observed between the L- and P/Q-type channels (\textit{P} = 0.037), while the N-type channel response was significantly smaller (\textit{P} < 0.001). Compared to the other two channel types, L-type VSCCs showed a stronger preference for the plateau waveform than the other 3 waveforms given. We have illustrated this difference (comparison between plateau and other waveforms within a channel type) on the figure using asterisks: \* \textit{P} < 0.05; ** \textit{P} < 0.01; *** \textit{P} < 0.001.
small steady drop in baseline, while the P/Q response was associated predominantly with APs at the beginning of the waveform (Figs 2A and 3). The N-type channel showed relatively little Ca\(^{2+}\) influx in response to the plateau-type waveform with current largely associated with the initial spike. The restriction of the current to the initial spike is probably attributed to channel’s closure due to insufficient depolarization (given the channel’s more positive activation curve) as well as voltage-dependent inactivation (Fig. 1C and D). Although the plateau waveform led to a considerably more sustained level of depolarization than the repetitive firing waveform, it produced only a 67% increase in current for the L-type channel and only 27% increase for the N-type channel (Fig. 2B). This observation suggests that APs and not necessarily large, potentially non-physiological plateau potentials or EPSPs are required to produce effective activation of L-type VSCCs. The ability of the L-type VSCC to become effectively activated by postsynaptic spiking is consistent with a recent report showing that APs alone are sufficient to induce late-phase long-term potentiation (LTP)-related cell signalling (Dudek & Fields, 2002). Interestingly, the difference in integrated current among the L-, P/Q- and N-type VSCCs in response to the complex waveforms (repetitive firing or plateau) was significantly larger than that expected from the response to more simple step depolarization (Figs 1B, 2A and B) indicating that unique channel properties emerge with the more physiological relevant waveforms permitting selective activation. Furthermore, marked temporal differences in the pattern of Ca\(^{2+}\) entry was observed between the channels with the complex waveforms. For example, significant Ca\(^{2+}\) entry only occurs during the first third of the 1 s stimulus for P/Q- and N-type channels (Fig. 2A).

In addition to the 100 Hz tetanic stimulation protocol, we also used a simulated theta rhythm in which the postsynaptic effect of 7.5 Hz presynaptic stimulation was determined. Under conditions with a strong synaptic conductance our model produced a voltage waveform with robust repetitive firing followed by Na\(^+\) channel inactivation and spike accommodation. With a weaker synaptic conductance only three APs were observed on the first two bursts and two APs on the third (Fig. 2A and B). Subsequent theta stimuli resulted in EPSPs only. In experiments on HEK 293 cells a significantly larger

![Figure 3. Cumulative plots of Ca\(^{2+}\) currents in response to complex waveforms](A, voltage commands used to evoke the VSCC currents in HEK 293 cells. The inset shows the expanded view of the first firing segment. Calibration bar is 10 ms. B, sample cumulative plots of Ca\(^{2+}\) currents (total charge) recorded from individual HEK 293 cells expressing L-, P/Q- or N-type VSCCs (using the same current traces as shown in Fig. 2A). Responses were normalized to a preceding step pulse as described in the Methods (and employed in Fig. 3B) to compare the total charge influx mediated by the different VSCC types. C, cumulative current plots normalized to the maximal value permitting a better comparison of response kinetics. Note for N- and P/Q-type channels charge influx was primarily associated with APs, \(\alpha_{1A}\) (P/Q-type), \(\alpha_{1B}\) (N-type) and \(\alpha_{1C}\) (L-type) subunits were expressed in combination with \(\beta_{1A}\) and \(\beta_{1A}\) subunits.)
response of the L-type VSCC (compared to the P/Q- and N-type VSCCs) was observed with the strong theta stimuli but not the weak stimuli (Fig. 2A and B). Inspection of the individual records revealed that the L-type Ca\(^{2+}\) current was largely associated with the APs and was rapidly and reversibly truncated during the Na\(^+\) channel inactivation (Figs 2A and 3C). In contrast, examination of the P/Q-type records (strong theta response) revealed an exponential reduction in the AP response (Fig. 2A) with each successive AP suggesting that an inactivation process was initiated that proceeded over the course of the additional stimuli. In response to the strong theta waveform the N-type channel current showed an initial rapid decrement in response associated with the first group of high-frequency spikes followed by recovery (within the same burst). A component of the reversible suppression of the N-type response was attributed to this channel’s relatively more positive activation voltage (\(V_{\text{th}} = +6\ mV \) at 2 mm CaCl\(_2\)). Presumably, the truncated action potentials would be of insufficient amplitude to fully activate the N-type channel.

To better evaluate differences in the time course of VSCC activity and to visualize slowly evolving steady inward currents (especially associated with the plateau waveform), we have plotted the cumulative Ca\(^{2+}\) current for the three channels examined (Fig. 3A and B). By normalizing the response of each channel to its total

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**Figure 4. \(\beta\)-Subunit expression differentially regulates the response of VSCCs to complex voltage waveforms**

Inactivation was assessed by placing a 5 ms square pulse (to +25 mV) at the end of the train stimulus and comparing its response amplitude (tail Ca\(^{2+}\) current (\(I_{\text{Ca}}\) peak)) to the amplitude of the first action potential-induced current at 37°C in both the repetitive firing and plateau waveforms. A, sample traces showing the VSCC current in response to the depolarization waveforms with the 5 ms end-pulses. \(\alpha_{1A}\) (P/Q-type) and \(\alpha_{1C}\) (L-type) subunits were expressed in combination with \(\beta_{1B}\) or \(\beta_{2A}\) (as indicated) and \(\beta_{2D}\) subunits. B, group data showing the ratio of response amplitude (5 ms end-pulse response versus that to the first action potential). C, group data illustrating the normalized integrated current response for P/Q- and L-type channels expressing either \(\beta_{1B}\) or \(\beta_{2A}\); * \(P < 0.05\); ** \(P < 0.01\); *** \(P < 0.001\), comparison for a particular channel and waveform with either \(\beta_{1B}\) or \(\beta_{2A}\): L- (\(n = 9\)) and P/Q-type (\(n = 7\)).
integrated current (Fig. 3C) it was clear that P/Q- and N-type channels contributed the most during the first few 100 ms of the waveforms used. The responses of all three channels to the repetitive firing waveform were largely associated with the AP components and not the steady EPSP component. The use of strong theta burst stimuli indicated a diminishing role of P/Q- and N-type channels with each subsequent burst of a seven-burst train. The use of weak theta train revealed little difference between the three channel types.

Selective inactivation of P/Q- and N-type voltage sensitive Ca^{2+} channels in response to complex synaptic waveforms

L- and P/Q-type channels are both subject to voltage- and Ca^{2+}-dependent inactivation (Yue et al. 1990; Lee et al. 1999). It is possible that the selective response of the L-type VSCC to the repetitive firing waveforms is a result of P/Q- and N-type channels becoming preferentially inactivated. To test this hypothesis we used a large step depolarization (to 25 mV for 5 ms) at the end of the complex waveform to monitor inactivation (Fig. 4A). We observed that a considerably smaller fraction of the P/Q-type VSCCs was available for activation at the end of the waveform compared to the L-type (Fig. 4A and B). This result was consistent with preferential inactivation of P/Q-type VSCCs contributing to their relatively smaller response to complex voltage waveforms such as the repetitive firing stimulus or the plateau potentials.

To examine the issue of inactivation further, VSCC subunit combinations with reduced voltage-dependent inactivation were expressed (Stotz & Zamponi, 2001). Expression of the \( \alpha_{1a}/\beta_{2a}/\alpha_{2d} \) subunit combination markedly attenuated the inactivation of P/Q-type channels in response to a 1 s step depolarization to 0 mV at 37°C (fast decay \( \tau \) lengthens from 22.6 ± 1.8 ms, \( n = 31 \) to 72.8 ± 11.4 ms, \( n = 5 \), \( P < 0.001 \); steady-state current level increases from 2.7 ± 0.4%, \( n = 8 \) to 32.5 ± 2.6% of the peak amplitude, \( n = 5 \), \( P < 0.001 \); see Table 2). Under these conditions we observed that the P/Q-type VSCC exhibited a very strong response to the complex waveforms (Fig. 4A).

Figure 5. Differential recovery of VSCCs from inactivation in response to a gap in AP firing

A, the left column shows the Ca^{2+} current in response to the repetitive firing waveform (see Fig. 4A for original), with four action potentials removed. During the AP gap (at −45 to −50 mV) the L-type VSCC current recovered while the P/Q- and N-type continued to inactivate. On the right column, the membrane potential during the gap was hyperpolarized to −140 mV leading to marked acceleration of P/Q- and N-type channel recovery from inactivation. B, group data illustrating differences in recovery between the channel types in response to the 4-AP gap. Values shown are ratio of the average amplitude of the Ca^{2+} current elicited by three action potentials after the gap versus average Ca^{2+} current amplitude for three action potentials before the gap. For L-type VSCCs, both the normal gap waveform (left column) and the −140 mV hyperpolarized gap waveform have significantly increased ratios (1.07 ± 0.03, \( n = 9 \) and 1.46 ± 0.16, \( n = 4 \) respectively) compared to normal repetitive firing waveform (0.99 ± 0.01, \( n = 9 \)). For P/Q-type Ca^{2+} channels, removal of 4 APs did not significantly reduce the progressive inactivation (ratios: 0.82 ± 0.04, \( n = 5 \) and 0.87 ± 0.01, \( n = 5 \) for the regular repetitive waveform and normal gap waveform respectively). Hyperpolarization to −140 mV greatly accelerated the recovery of the P/Q-type channel from inactivation (3.26 ± 0.35, \( n = 8 \)). This enhanced recovery is significantly more than that observed in the L-type channels (\( P = 0.0062 \)). Similar to the P/Q-type channels, inactivation of the N-type was not attenuated in normal gap waveform (0.73 ± 0.02, \( n = 6 \)) as compared to that in regular repetitive waveforms (0.75 ± 0.02, \( n = 8 \)). The ratio for hyperpolarized gap waveform in N-type channels was also significantly increased (2.01 ± 0.28, \( n = 2 \)). Experiment temperature was 37°C. Asterisks indicate comparisons to the normal repetitive firing waveform among the same channel type: * \( P < 0.05 \); ** \( P < 0.01 \); *** \( P < 0.001 \). \( \alpha_{1a} \) (P/Q-type), \( \alpha_{1b} \) (N-type) and \( \alpha_{1c} \) (L-type) subunits were expressed in combination with \( \beta_{1a} \) and \( \alpha_{2d} \) subunits.
In fact the integrated current responses from the P/Q-type (\(a_{1A}/b_{2A}/a_{2d}\)) exceeded those of the L-type VSCC indicating that the differential expression of \(b\) subunits and regulation of \(a\) subunits by alternative splicing (Bourinet et al. 1999) can have a significant physiological impact on channels expressed. Consistent with the L-type channel having predominantly Ca\(^{2+}\)-dependent inactivation, expression of L-type channels containing \(b_{2A}\) did not appreciably alter the integrated currents obtained (as compared to the P/Q-type channel). To examine the role of Ca\(^{2+}\)-dependent inactivation of L-type channels we substituted Ba\(^{2+}\) for Ca\(^{2+}\) and found a marked increase in the normalized integrated current when complex waveforms were used (data not shown).

The continuing inactivation observed in P/Q- and N-type channels during the repetitive firing waveform may be partly attributed to the difference in inactivation voltage dependence between L-type and P/Q- or N-type channels, with the L-type channel having a more depolarized inactivation profile. In addition, work by Yue and colleagues suggest (Patil et al. 1998) that P/Q- and N-type channels can become inactivated during a relatively hyperpolarized interval between successive depolarizing stimuli. According to this mechanism the channels can enter inactivated states from a closed state thus membrane potentials that lead to inactivation need not necessarily first activate the channel. This form of inactivation is termed intermediate closed-state inactivation and might help explain why P/Q-type channels are selectively inactivated by complex voltage stimuli that contain relatively brief periods of AP depolarization (sufficient to activate the channel) when compared to sustained step depolarization. A similar phenomenon also occurs with Na\(^{+}\) channels and contributes to activity-dependent alterations in dendritic spiking (Colbert et al. 1997; Mickus et al. 1999). In the case of our VSCC stimuli, the depolarized AP peaks in combination with more moderate EPSPs may be ideal for producing selective inactivation of P/Q- and N-type Ca\(^{2+}\) channels. To test this idea we removed four successive APs from the simulated repetitive firing waveform to determine whether inactivation of P/Q-type channels would proceed in the absence of AP stimulation. Our results indicate that the four spikes had little effect on the inactivation of subsequent AP-evoked Ca\(^{2+}\) currents (Fig. 5A). To quantify this effect we measured the average peak current attributed to the three spikes preceding the gap and three after the gap. In the case of the P/Q-type channel a significant reduction in the current amplitude associated with the latter three spikes was observed (_13±1\%, \(P<0.05\) paired \(t\) test, \(n=5\)) following the four AP gap; while removal of the APs led to an increase in the L-type VSCC response (7±3\%, \(P<0.05\) paired \(t\) test, \(n=9\)). Similar to the P/Q-type channels, inactivation of the N-type was not attenuated by placing a four AP gap in the waveform as compared to the regular repetitive firing waveforms (_27±2\%, \(n=6\) without and _25±2\%, \(n=8\) with the gap; Fig. 5B). Consistent with the idea that voltage-dependent inactivation was important for the apparent difference between the channels, insertion of a hyperpolarized interval (_140mV) during the AP gap led to a profound facilitation (>100\% increase) of the AP-evoked Ca\(^{2+}\) current mediated by the P/Q- and N-type channels for several 100ms after the gap (Fig. 5A and B). Based on previous work the hyperpolarized interval would be expected to speed recovery from inactivation (Patil et al. 1998). Although hyperpolarization to _140 mV is not physiological, it nonetheless illustrates the importance of the steady-state membrane potential during the interval between spikes within short trains in determining both the

<table>
<thead>
<tr>
<th>Temperature</th>
<th>(\tau_1) (ms)</th>
<th>(\tau_1) (%)</th>
<th>(\tau_2) (ms)</th>
<th>(\tau_3) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>22°C</td>
<td>29.6 ± 1.9</td>
<td>45.3 ± 2.3</td>
<td>301.5 ± 17.6</td>
<td>40.8 ± 2.6</td>
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<td></td>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=8)</td>
<td>(n=8)</td>
</tr>
<tr>
<td>P/Q</td>
<td>17.8 ± 1.4</td>
<td>72.4 ± 2.1</td>
<td>207.5 ± 16.2</td>
<td>18.5 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>(n=23)</td>
<td>(n=23)</td>
<td>(n=22)</td>
<td>(n=21)</td>
</tr>
<tr>
<td>P/Q</td>
<td>22.6 ± 1.8</td>
<td>75.2 ± 2.2</td>
<td>172.8 ± 16.0</td>
<td>19.0 ± 3.0</td>
</tr>
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<td></td>
<td>(n=31)</td>
<td>(n=26)</td>
<td>(n=26)</td>
<td>(n=26)</td>
</tr>
<tr>
<td>N</td>
<td>18.1 ± 1.6</td>
<td>77.0 ± 3.9</td>
<td>270.2 ± 45.7</td>
<td>15.1 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>(n=15)</td>
<td>(n=14)</td>
<td>(n=15)</td>
<td>(n=14)</td>
</tr>
</tbody>
</table>

All of the decay constants (\(\tau\)) were significantly reduced by increased temperature (\(P<0.001\)). \* P/Q-type currents decay with a single exponential component at room temperature. \% refers to the percentage of the peak current that was described by each exponential component. \(\alpha_\alpha\) (P/Q-type), \(\alpha_\infty\) (N-type) and \(\alpha_\infty\) (L-type) subunits were expressed in HEK 293 cells in combination with \(\beta_\alpha\) or \(\beta_\delta\) and \(\beta_{2A}\) subunits.
number of channels available for activation and their recovery from previous inactivation.

The data presented above suggest that the selective response of the different channel types to the repetitive firing waveform could be attributed to the presence of the EPSP component that contributes to preferential voltage-dependent inactivation of P/Q- and N-type channels. To further evaluate this we contrasted the effect of EPSP removal for L-, P/Q- and N-type VSCCs (see example of P/Q data in Fig. 6A). Removal of the EPSP component from the stimulus waveform (leaving only APs delivered from −70 mV) resulted in a significant decrease in the integrated Ca$^{2+}$ current via the L-type VSCC and an increase in integrated Ca$^{2+}$ current via the P/Q- and N-type channels (Fig. 6A and B). Without the EPSP component, the integrated L-type current (38.5 ± 2.4 pC nA$_{peak}^{-1}$, n = 12) was still significantly larger than that mediated by P/Q- (28.5 ± 3.6 pC nA$_{peak}^{-1}$, n = 9; P < 0.05, Fig. 6B) or N-type channels (17 ± 3 pC nA$_{peak}^{-1}$, n = 8; P < 0.001, compare with EPSP-containing value 11 ± 2 pC nA$_{peak}^{-1}$, n = 8), suggesting that EPSPs in combination with APs produce selective P/Q- and N-type channel inactivation and thus contribute to the privileged response of the L-type VSCC to complex waveforms.

Since the recovery of VSCCs from inactivated states is accelerated by hyperpolarization (Kass & Sanguinetti, 1984; Gutnick et al. 1989; Patil et al. 1998) (see Fig. 5), we edited our repetitive firing waveform so that the APs would be elicited from an extremely negative potential (−110 mV). APs elicited from this holding potential were associated with significantly larger normalized Ca$^{2+}$ currents for the P/Q- and N-type channels (Fig. 6B). For the P/Q-type channel, hyperpolarization to −110 mV largely blocked inactivation as measured by the ratio of the Ca$^{2+}$ current response (last/first AP response; 0.84 ± 0.05, n = 5). In contrast, the ratio for the normal repetitive firing waveform with approximately a −50 mV interspike potential was significantly lower (0.11 ± 0.01, n = 9; P < 0.001, see Fig. 2A for an example). A repetitive firing waveform lacking EPSPs and with a −70 mV interspike potential had an intermediate ratio when P/Q-type channels were expressed (0.52 ± 0.03, n = 9; P < 0.001). Hyperpolarization also increased the currents mediated by the N-type channel, although to a lesser extent (ratio of the last AP to first: 0.27 ± 0.04, n = 6 for −110 mV no EPSP; 0.05 ± 0.01, n = 11 for the normal repetitive firing waveform; 0.15 ± 0.02, n = 8 for −70 mV no EPSP). The ability to modulate the degree of VSCC inactivation by the interspike membrane potential provides additional data.

Figure 6. Differential effect of EPSP removal from complex waveforms on L-type versus P/Q- and N-type VSCCs

A, example of Ca$^{2+}$ currents mediated by P/Q-type VSCCs stimulated by the repetitive firing voltage waveform without the EPSP component (centre panel) at 37°C. Progressive inactivation of the VSCC current in response to AP stimuli was reduced by removal of EPSPs (see Fig. 2A for control example with both EPSPs and APs). Hyperpolarization to −110 mV (AP amplitude increased so the peaks are at the same membrane potential) produced a further attenuation of inactivation associated with the AP stimuli. B, group data showing the mean normalized integrated VSCC current in response to repetitive firing waveforms with different interspike membrane potentials (−70 or −110 mV for N- and L-type VSCCs). α1a (P/Q-type), α1b (N-type) and α1c (L-type) subunits were expressed in HEK 293 cells in combination with β1a and α2δ subunits. * P < 0.05; ** P < 0.01; *** P < 0.001, comparison by ANOVA between with EPSP (typical repetitive firing waveform) and −70 mV no EPSP or −110 mV no EPSP, the results shown are from n = 4–12 cells for each group).
supporting preferential voltage-dependent inactivation as contributing to the waveform preference we observe. In contrast to the P/Q- and N-type channels, the L-type VSCC was relatively unaffected by the interspike membrane potential (ratio of the last AP to first: $0.79 \pm 0.09, n = 4$ for $-110 \text{ mV}$; $0.45 \pm 0.03, n = 10$ for normal repetitive; $0.62 \pm 0.04, n = 13$ for $-70 \text{ mV}$).

**Recovery from inactivation at physiological temperature**

The use of complex waveforms that lack EPSPs indicated that some of the difference in integrated current between L-, P/Q- and N-type channels was due to differences in inactivation triggered by steady depolarization. This is expected since the steady-state inactivation curves were markedly different between the channels (L-type channels are $30 \text{ mV}$ more positive; Fig. 1D). In addition to reduced inactivation, some of the preferential response of the L-type channel could be attributed to faster recovery from inactivation. To further examine this issue we produced repetitive firing voltage waveforms (containing both EPSPs and APs) with depolarizing test pulses (5 ms step to $+25 \text{ mV}$) given at various intervals after the conditioning train (Fig. 7A and B). The amplitude of the test pulses was compared to that of a pulse given before the repetitive firing waveform. Comparison of the three channel types indicated that the P/Q-type channel showed markedly slower recovery from inactivation than the L- or N-type channels (Fig. 7B). Using a different inactivation protocol with 1 s of sustained depolarization to 0 mV we found that a slow component of recovery ($> 1 \text{ s, } \sim 25\%$) was also observed for all three channel types.

**DISCUSSION**

In this study we have focused on the activation of high threshold Ca$^{2+}$ channels by physiologically relevant voltage waveforms. Our emphasis has been on contrasting the response of L-, P/Q- and N-type channels. We have expressed L-type channels composed of $\alpha_{1C}$ subunits that recent data from hippocampal neurons suggest are predominantly involved in coupling depolarization to activation of gene transcription factor cAMP-response element binding protein (CREB)-dependent gene expression (Weick *et al.* 2003). It should be stressed that L-type channels can also be composed of $\alpha_{1D}$ subunits that have been shown to constitute a component of the L-type current in neurons (Hell *et al.* 1993). However, $\alpha_{1D}$ (in contrast to $\alpha_{1C}$) does not appear to couple to intracellular effectors such as PDZ-domain-containing proteins (Weick *et al.* 2003) that are required for efficient L-type channel-dependent stimulus–transcription coupling and therefore we have focused our study on $\alpha_{1C}$ containing channels.

Our data show that at physiological temperature, L-, P/Q- and N-type VSCCs all exhibit markedly enhanced activation, inactivation and peak current amplitude compared to room temperature. At room temperature all three channels require a significantly longer duration of

![Figure 7. Recovery from inactivation induced by complex voltage waveforms](image-url)
depolarization to become activated than that required at 37°C. Thus, at reduced temperature (22°C) it is possible that conditions that alter AP duration such as the modulation of K channels would change both the degree and duration of channel activation (Sabatini & Regehr, 1997). The rapid activation of L-type VSCCs with stimuli resembling single APs we observed is consistent with previous pharmacological data showing a role for the L-type channel in AP-mediated Ca transient (Christie et al. 1995a). The effective activation of L-type VSCCs by APs alone (without a requirement for EPSPs) indicates that specific frequencies of postsynaptic spiking may be an effective means of activating events associated with late phase long-term potentiation, consistent with recent findings (Dudek & Fields, 2002). However, it is likely that EPSPs in combination with APs are required to tag and strengthen specific synapses (Deisseroth et al. 1996; Frey & Morris, 1997). Although EPSPs may be required for triggering local chemical events (perhaps through NMDA-receptor-mediated Ca influx) they are actually relatively inefficient at activating VSCCs even when of large amplitude. To address this more quantitatively we asked what was the best way to activate VSCCs (measured by integrated current over 1 s if given the ability to deliver 100 ms of depolarization from a -70 mV holding potential in different ways over a period of up to 1 s (data not shown)). For example, what provides more integrated VSCC current, 100 1 ms steps to 0 mV (given over 1 s) or 100 ms of continuous depolarization? Other combinations are also possible 10 10 ms steps or 333 0.3 ms steps. Surprisingly steps resembling actual action potentials (half-widths of ~1 ms) were more effective at activating L- and P/Q-type channels. The reasons for this are easy to understand and include: little inactivation associated with 1 ms pulses and a high proportion of the current appearing as a tail current (having a larger driving force). Pulses of shorter duration (0.3 ms) are expected to be less effective since they would fail to fully activate the channels (Fig. 1F). Thus the activation and inactivation rates for high threshold VSCCs are tuned to the properties (amplitude and shape) of the AP and not necessarily long lasting EPSPs.

Our data indicating that APs are the most effective means of activating recombinant L-type VSCCs would seem to be in contradiction to recent data from us and others showing that L-type channels have a preference for large EPSPs over APs (Nakazawa & Murphy, 1999; Mermelstein et al. 2000). There are several potential explanations for this including the fact that the EPSPs used in these studies were prolonged step depolarizations to -30 to -15 mV and were not necessarily physiologically relevant waveforms. Given the steady-state inactivation curves of the L-, P/Q- and N-type channels (Fig. 1), it is possible that the waveforms selectively inactivated P/Q- and N-type channels. Furthermore, these waveforms only contained a single AP as opposed to 40–50 spikes contained in some of the waveforms used in this study. Previous observations that are more difficult to reconcile with the current data are why L-type VSCCs mediate only a relatively small fraction of the Ca response to single APs (Nakazawa & Murphy, 1999; Mermelstein et al. 2000). Potentially this finding can be explained if the relative abundance as well as the waveform preference of L-type versus other VSCCs is considered. For example, if only 20% of the VSCCs are L-type (defined by peak current amplitude in response to a step, as in Fig. 1), then assuming equal activation of all VSCC types we would expect 20% of the AP-evoked Ca transient to be blocked by a (L-type) dihydropyridine antagonist. In the case of the complex waveforms (such as the repetitive firing one we used) a factor related to waveform preference needs to be considered when evaluating the responses between channel types to different waveforms. Assuming the L-type has an abundance of only 20%, but results in an integrated current that is three times larger (waveform preference factor) with the repetitive firing waveform relative to the P/Q- and N-type, the responses to complex waveforms can be estimated as follows: 0.20 L-abundance × 3.0 L-preference = 0.6 L-response; versus 0.8 non-L-abundance × 1.0 non-L-preference = 0.8 non-L-response. Therefore addition of an L-type VSCC specific dihydropyridine antagonist could block 43% of the repetitive firing induced Ca transient (fraction L = 0.6 L/(1.4 total L + non-L)), but only 20% of the single AP response similar to previous reports using mixed populations of channels (Nakazawa & Murphy, 1999; Mermelstein et al. 2000).

Although closely functionally related channels such as the P/Q-type (with the inactivating B1B subunit expressed) respond similarly to the L-type VSCC when given 1 s step depolarization, they are selectively inactivated in response to more physiological waveforms, providing a mechanism to discriminate different voltage waveforms created by unique synaptic stimuli. The preferential response of the L-type channel to complex synaptic bursting was attributed to the P/Q- and N-type channels containing Ca channel B subunits that support voltage-dependent inactivation. Forebrain neurons have been reported to predominantly express the B1B isoform suggesting that P/Q-, N-type and other high threshold VSCCs that are subject to voltage-dependent inactivation would be selectively inactivated by the complex waveforms we have used (Ludwig et al. 1997). The voltage-dependent inactivation of the P/Q- and N-type channels were facilitated by steady depolarization in the form of EPSPs. In contrast, removal of EPSPs and holding the cell at very negative potentials (~110 mV) completely blocked the inactivation process. These findings highlight important differences for regulation of these channels in dendritic and axonal compartments.
where large differences in interspike membrane potential may be present. In the axon the P/Q- and N-type channels would not experience EPSPs and would have a more negative interspike membrane potential greatly reducing inactivation with trains of stimuli and thus increasing the reliability of transmission. In contrast, in the soma and dendrites EPSPs would help to inactivate non-L-type VSCCs (P/Q and N) and leave a response mediated predominantly by L-type channels. For L-type VSCCs that are subject to primarily Ca\(^{2+}\)-dependent inactivation, the local intracellular Ca\(^{2+}\)-handling environment is likely to influence waveform preference and could account for why sustained depolarization (1 s step to 0 mV) leads to relatively more L-type VSCC inactivation than the repetitive AP/EPSP waveform. Given that the various classes of VSCCs have different kinetics and mechanisms of inactivation it is tempting to imagine scenarios in which particular VSCCs are tuned to specific stimuli based on their inactivation and recovery kinetics. The ability of relatively low amplitude steady depolarization (i.e. to −55 mV) to influence the number of P/Q- and N-type channels available for activation without actually activating them provides a powerful mechanism for previous experience to modulate channel function. In contrast, L-type channels would be relatively insensitive to small voltage changes. Since P/Q- and N-type channels exhibit greatly accelerated recovery from inactivation in response to hyperpolarization, it is possible that if the hyperpolarized interval between bursts were longer than the recovery time these channels could be tuned to a particular frequency if given hyperpolarized intervals that are longer than recovery time. The presence of slow Ca\(^{2+}\)-activated K\(^+\) channels might provide hyperpolarization of sufficient duration and magnitude (to −80 mV) to relieve a significant amount of P/Q- and N-type inactivation. Alternatively, other factors within bursting voltage waveforms such as the presence of slow Na\(^+\) channel inactivation that lead to spikes of diminishing amplitude would favour the activation of L- and P/Q-type channels which are activated at more negative potentials than N-type channels. This phenomenon would be particularly apparent in dendrites where slow Na\(^+\) channel inactivation is most apparent (Colbert et al. 1997; Mickus et al. 1999). In addition to AP amplitude, other mechanisms may be at work in dendrites to result in more selective VSCC activation with trains of stimuli. For example, although high-voltage activated Ca\(^{2+}\) channels are similarly activated with APs of brief duration (< 1 ms), the longer AP duration and greater EPSP amplitude found in distal dendrites (Stuart et al. 1997a; Magee & Cook, 2000) may promote the selective inactivation of P/Q- and N-type channels. Although we have stressed that L-type VSCCs can be differentially tuned to voltage waveforms we should point out that not all neurons or their associated subcellular components have a large fraction of L-type current (El Manira & Bussieres, 1997; Barnes-Davies et al. 2001). In these neurons different β subunit expression may determine whether P/Q- and N-type channels can respond to complex waveforms similar to the ones we have used.

In conclusion, we propose that in response to complex synaptic bursting waveforms of long duration P/Q- and N-type channels undergo selective inactivation leading to a Ca\(^{2+}\) current mediated predominantly by L-type channels providing a mechanism that works in combination with local chemical events (Deisseroth et al. 1996; Dolmetsch et al. 2001; Weick et al. 2003) to couple these channels to specific tasks such as activity-dependent gene expression (Nguyen et al. 1994). However we emphasize that these results from heterologously expressed Ca\(^{2+}\) channels need to be repeated in actual hippocampus neurons since a variety of factors including α, splice variants, β subunit expression, and interacting proteins can potentially influence channel kinetics. In addition, VSCC behaviour can even vary within a neuron as channels on neuritic branches can have different properties from those expressed at the soma (Egorov et al. 1999; Li et al. 2001). Thus it is thus possible that subcellular differences in VSCC structure combined with compartment specific changes in voltage also contribute to selective coupling between synaptic stimuli and VSCC activity.

REFERENCES


APPENDIX

Table 3. Distribution of ionic channels in the NEURON model

<table>
<thead>
<tr>
<th>Channel</th>
<th>Dendrite (distal) mS cm⁻²</th>
<th>Dendrite (proximal) mS cm⁻²</th>
<th>Soma mS cm⁻²</th>
<th>Hillock mS cm⁻²</th>
<th>Axon mS cm⁻²</th>
<th>Source of model and conductance level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na</td>
<td>15</td>
<td>15</td>
<td>300</td>
<td>15</td>
<td></td>
<td>(Destexhe et al., 1994; Mainen &amp; Sejnowski, 1998) Maximum conductance on the dendrites was adjusted to obtain proper excitability of the model cell (Lipowsky et al., 1996)</td>
</tr>
<tr>
<td>NaP</td>
<td>0.02</td>
<td>0.05</td>
<td>0.1</td>
<td>0.1</td>
<td></td>
<td>(Mainen &amp; Sejnowski, 1998)</td>
</tr>
<tr>
<td>K_a</td>
<td>3 mS cm⁻²</td>
<td>3</td>
<td>60</td>
<td>3</td>
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<td>(Hoffman et al., 1997) Adjusted to balance NaP at resting membrane potential (Mainen &amp; Sejnowski, 1998)</td>
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<tr>
<td>K_st</td>
<td>7 + 11*(distance from soma in μm/100) mS cm⁻²</td>
<td>7</td>
<td>7</td>
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<td></td>
<td>(Lipowsky et al., 1996) (Sah &amp; Bekkers, 1996)</td>
</tr>
<tr>
<td>K_m</td>
<td>0.2 mS cm⁻²</td>
<td>0.2</td>
<td>0.2</td>
<td>0.4</td>
<td></td>
<td>(Lipowsky et al., 1996) (Mermelstein et al., 2000) Adjusted to represent different levels of synaptic stimulation (Destexhe et al., 1994)</td>
</tr>
<tr>
<td>NaP</td>
<td>0.2 mS cm⁻²</td>
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<td></td>
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<tr>
<td>Ca_k</td>
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<td></td>
<td></td>
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<td>NMDA</td>
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<tr>
<td>AMPA</td>
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</tbody>
</table>

For simplicity there are three synapses, two on the distal dendrites and one on proximal dendrite. The synaptic conductances (NMDA and AMPA) were adjusted in simulations as needed. The above distribution of ionic channels is for nonbursting cells. For bursting cells, there is more NaP (persistent Na⁺ channels) (0.1 mS cm⁻² on proximal dendrites, 0.05 mS cm⁻² on distal dendrites).


Acknowledgements

We thank Dr Terry Snutch for the gift of VSCC clones, the N-type VSCC stable cell line and for technical advice. We thank Brian Christie, Yo Otsu and Lynn Raymond for comments on the manuscript. This work was supported by an operating grant from the CIHR of Canada (MOP-12675). T.H.M. is a CIHR investigator and a MSFHR Senior Scholar.

Supplementary material

The online version of this paper can be found at:

DOI: 10.1113/jphysiol.2003.051110

and contains material entitled:

Digital versions of the voltage clamp stimulus waveforms used in the paper (repetitive, plateau, strong theta and weak theta; see Fig. 2).