Interactive Effects of the GABAergic Modulation of Calcium Channels and Calcium-Dependent Potassium Channels in Lamprey

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Tegnérl, Jesper and Sten Grillner. Interactive effects of the GABAergic modulation of calcium channels and calcium-dependent potassium channels in lamprey. J. Neurophysiol. 81: 1318–1329, 1999. The GABAergic-mediated modulation of spinal neurons in the lamprey is investigated in this study. Activation of GABA receptors reduces calcium currents through both low- (LVA) and high-voltage activated (HVA) calcium channels, which subsequently results in the reduction of the calcium-dependent potassium (KCa) current. This in turn will reduce the peak amplitude of the afterhyperpolarization (AHP). We used the modulatory effects of GABA receptor activation on N-methyl-D-aspartate (NMDA)-induced, TTX-resistant membrane potential oscillations as an experimental model in which to separate the effects of GABA receptor activation on LVA calcium channels from that on KCa channels. We show experimentally and by using simulations that a direct effect on LVA calcium channels can account for the effects of GABA receptor activation on intrinsic membrane potential oscillations to a larger extent than indirect effects mediated via KCa channels. Furthermore, by conducting experiments and simulations on intrinsic membrane potential oscillations, we find that KCa channels may be activated by calcium entering through LVA calcium channels, providing that the decay kinetics of the calcium that enters through LVA calcium channels is not as slow as the calcium entering via NMDA receptors. A combined experimental and computational analysis revealed that the LVA calcium current also contributes to neuronal firing properties.

INTRODUCTION

Neuromodulators affect the operation of neural networks (Grillner et al. 1995; Marder and Calabrese 1996). A neuromodulator acts as a rule via several ionic mechanisms. It is therefore of interest to identify the role of the separate mechanisms and determine the summed effects at the neuronal and network level. We combine physiological experiments with computer simulations to investigate the neuromodulatory action of GABA receptor activation on the neuronal level in lamprey (Matsushima et al. 1993). We focus on the interactive effects between Ca2+ and KCa channels in spinal neurons.

The membrane properties of neurons in the lamprey spinal cord were characterized in some detail (Buchanan 1982, 1993; Buchanan and Grillner 1987; Rovainen 1983; Sigvardt et al. 1985; Wallén and Grillner 1987). Both low voltage-activated (LVA) and high-voltage-activated (HVA) calcium channels are present (Bacska et al. 1995; Christenson et al. 1993; El Manira and Bussières 1997; Grillner et al. 1998; Matsushima et al. 1993). The calcium entering through HVA calcium channels during an action potential (AP) activates apamin-sensitive KCa channels (KCaAP). These channels are activated during the postsynaptic afterhyperpolarization (AHP) and determine the degree of spike frequency adaptation (El Manira et al. 1994; Gustafsson 1974; Hill et al. 1992; Meer and Buchanan 1992; Wallén et al. 1989), whereas LVA calcium channels allow neurons to fire rebound spikes (Matsushima et al. 1993; Tegnérl et al. 1997a). Subtypes of these channels can be influenced by modulators such as 5-hydroxytryptamine (El Manira et al. 1997; Wallén et al. 1989), dopamine (McPherson and Kemnitz 1994; Schotland et al. 1995), and GABA (El Manira and Bussières 1997; Matsushima et al. 1993). These modulators either reduce the conductance through KCa channels directly or reduce it indirectly through an effect on Ca2+ channels.

GABA-immunoreactive neurons are present in the lamprey spinal cord (Brodin et al. 1990). GABA receptors act via G proteins in the lamprey spinal cord (Alford and Grillner 1991; Alford et al. 1991; El Manira and Bussières 1997; Matsushima et al. 1993). The effects include 1) a reduction of LVA calcium currents (Matsushima et al. 1993; Tegnérl et al. 1997a) leading to 2) a decreased postsynaptic rebound depolarization (Matsushima et al. 1993; Tegnérl et al. 1997a) and 3) a reduced current through HVA calcium channels (Matsushima et al. 1993), which decreases 4) the amplitude of the apamin-sensitive KCa channels (KCaAP) and thereby the peak amplitude of the slow AHP (Matsushima et al. 1993; Tegnérl et al. 1997a). GABA receptor activation also results in presynaptic inhibition, 5) which subsequently decreases inhibitory and excitatory postsynaptic potentials from premotor interneurons (Alford and Grillner 1991; Alford et al. 1991).

The objective of this study was to investigate the relative importance of the modulatory effect of GABA receptor activation on LVA calcium channels and its indirect effect on KCaAP calcium channels brought about by its direct action on HVA calcium channels. We examined whether calcium entry through LVA Ca2+ channels activates KCa channels and what influence LVA calcium currents have on neuronal firing properties. To this end, we used intracellular recordings to examine the effect of GABA receptor activation on intrinsic N-methyl-D-aspartate (NMDA)-induced, TTX-resistant membrane potential oscillations (NMDA-induced oscillations). This analysis was extended with computer simulations to separate the effects of Ca2+ channels from those of KCa channels. We used the pacemaker oscillations as a model system for how calcium and...
calcium-dependent properties interact. Thus we do not address the possible role of the NMDA-induced oscillations in the swimming rhythm.

Some of the results were reported previously in abstract form (Tegnér et al. 1992, 1997b).

METHODS

Experiments

PROTOCOL. Experiments were performed with adult lampreys Petromyzon marinus \((n = 2)\) and Ichthyomyzon unicuspis \((n = 11)\). The animals were anesthetized with tricaine methane sulfonate (MS222, 100 mg/l). The spinal cord was isolated in cooled physiological Ringer and pinned down in a Sylgard-lined chamber (Rovainen 1974; Wallén et al. 1985). The preparation was perfused with oxygenated saline containing (in mM) 91 NaCl, 2.1 KCl, 2.6 CaCl\(_2\), 1.8 MgCl\(_2\), 20 NaHCO\(_3\), and 4 glucose (pH 7.4), with the temperature held at \(-9^\circ\text{C}\). Intracellular recordings were performed with sharp microelectrodes. Discontinuous current clamp (DCC) was used to control the membrane potential. Drugs were added directly to the saline: NMDA (Tocris Neuramin), TTX, baclofen, and 2-OH saclofen (Sigma) (Grillner et al. 1981). Dorsal cells and edge cells were not included in this study. Neurons exhibiting pacemaker oscillations were not identified because TTX was used here. Lateral interneurons do not oscillate in NMDA and TTX (Wallén and Grillner 1987). Data were acquired with a personal computer (486 PC type) with AD/DA interface programs (AXOTAPE, Axon Instruments).

ANALYSIS OF EXPERIMENTAL DATA. The threshold for detecting the onset and offset of the plateau phase was identified as indicated in Fig. 1. The threshold was manually set between the average plateau potential and the trough potential. No detectable difference in the results was obtained when this procedure was repeated with the same trace. That the oscillation period is large and the slope of the voltage curve is steepest around the threshold accounts for the finding that the results are not sensitive to exactly where the threshold is taken. The following parameters were analyzed (Fig. 1): cycle duration (peak-to-peak duration), the duration of the plateau, and the hyperpolarized phase, with the hyperpolarized phase defined as the difference in time between the cycle duration and plateau duration. The plateau proportion was calculated as the ratio between the duration of the plateau and the cycle duration. The peak amplitude was defined as the maximum voltage of the first peak during the plateau phase. The trough potential refers to the lowest potential during the hyperpolarized phase.

The first spike interval was measured and calculated with DATAPAC (Axon Instruments), and the Student’s \(t\)-test was used.

Computer simulations

SINGLE CELL MODEL. We used a compartmentalized model neuron (Koch and Segev 1989; Rall 1977) with Hodgkin–Huxley-type membrane properties (Hodgkin and Huxley 1952). The neuron is represented in the simulations by a soma, a small initial segment compartment, and a dendritic tree of three compartments connected in series to the soma compartment (Grillner et al. 1988; Wallén et al. 1992). Parameters of the cell model were previously matched to the properties of lamprey spinal neurons (Brodin et al. 1991; Ekeberg et al. 1991; Trävén et al. 1993). The soma is equipped with Na\(^+\), K\(^+\), and calcium channels of both HVA and LVA type (Tegnér et al. 1997a). Calcium-dependent potassium channels (K\(_{\text{Ca}}\)) are also included in the soma of the model. The initial segment has Na\(^+\) and K\(^+\) conductance. In addition to the passive properties, the three dendritic compartments also have ion channels to represent input synapses. Inhibitory synapses are located on the dendritic compartment adjacent to the soma, whereas excitatory synapses are placed on the second dendritic compartment (Ekeberg et al. 1991; Russell and Wallén 1983). Excitatory and inhibitory synaptic effects are modeled as conductance changes in the dendritic compartment. Excitatory synapses have voltage-dependent NMDA receptor channels as well as fast \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid/kainate synapses, both of which are saturating, accounting for the fact that the synaptic conductance cannot grow without limit as the firing frequency of the presynaptic cell increases (Trävén et al. 1993).

MODEL OF INTRACELLULAR CALCIUM AND INTERACTION WITH K\(_{\text{Ca}}\) CHANNELS. Like most other neurons lamprey spinal neurons exhibit spike frequency adaptation caused by opening of K\(_{\text{Ca}}\) channels during long-lasting activation. During continuous firing the intracellular calcium level increases (Bacsakai et al. 1995), leading to activation of a K\(_{\text{Ca}}\) current. It was therefore important to model intracellular calcium dynamics in addition to the K\(_{\text{Ca}}\) channels. Intracellular calcium is modeled by two different “intracellular pools.” A C\(_{\text{Ca,AP}}\) pool represents the calcium entering during the AP, and a C\(_{\text{Ca,NMDA}}\) pool represents the calcium inflow through NMDA channels (Brodin et al. 1991; Ekeberg et al. 1991). The K\(_{\text{Ca}}\) channels activated by these two calcium pools are referred to as K\(_{\text{Ca,AP}}\) and K\(_{\text{Ca,NMDA}}\) channels, respectively. Both the C\(_{\text{Ca,AP}}\) and C\(_{\text{Ca,NMDA}}\) calcium pools have parameters for calcium ion influx and decay. The parameter values used in this study are based on those determined in an earlier study (Brodin et al. 1991). Moreover, the
C$_{\text{NMDA}}$ pool has a variable, ranging from zero to one, representing the Mg$^{2+}$ block of the channel. When the cell model was extended with an LVA calcium channel (Tegnér et al. 1997a) an intracellular calcium pool was included to represent the calcium entering via LVA calcium channels. We investigated whether experimental data are consistent with the notion that a KC$_a$ conductance could be activated by calcium entering via LVA-channels, thus referred to as KC$_{a\text{LVA}}$ channel. Moreover, the LVA pool has parameters for the intracellular calcium kinetics, which we investigated.

The LVA calcium current is modeled as a sodium-like model but with different kinetics and thus different parameter values describing the state variable for activation ($m$) and inactivation of the LVA calcium conductance ($h$) (Tegnér et al. 1997a). The LVA current is computed as

\[ I_{\text{LVA}} = (E_{\text{LVA}} - E_{\text{rest}})G_{\text{LVA}}m^a_h \]

Thus essentially, the net LVA conductance depends on the $m^a_hG_{\text{LVA}}$ factor.

The third calcium pool (Tegnér et al. 1997a) used in the current simulations for the LVA calcium is modeled as

\[ \frac{\text{d}[\text{Ca}_{\text{LVA}}]}{\text{dt}} = (E_{\text{LVA}} - E_{\text{rest}})\rho_{\text{LVA}}m^a_h - \delta_{\text{LVA}}[\text{Ca}_{\text{LVA}}] \]

where the two constants $\rho_{\text{LVA}}$ and $\delta_{\text{LVA}}$ are the rates of calcium ion influx and decay, respectively. We also investigated the possibility that the calcium inflow through LVA calcium channels also contributes to the $I_{\text{K(Ca)}}$ current. The expression for the net current through the KC$_a$ channels with an LVA calcium channel included amounts to the following three components

\[ I_{\text{K(CaHVAM)}} = (E_K - E)G_{\text{K(CaHVAM)}[\text{CaHVAM}]} \]

\[ I_{\text{K(CaNMDA)}} = (E_K - E)G_{\text{K(CaNMDA)}[\text{CaNMDA}]} \]

\[ I_{\text{K(CaLVA)}} = (E_K - E)G_{\text{K(CaLVA)}[\text{CaLVA}]} \]

We used the same value for $E_K$ (−80 mV) as a first approximation in all three cases.

**RESULTS**

**Effects of GABA$_B$ receptor activation on NMDA-induced, TTX-resistant, membrane potential oscillations**

GABA$_B$ receptor activation reduces the calcium current entering via HVA and LVA calcium channels (Matsushima et al. 1993). The amplitude of the AHP following an AP is reduced because of the reduction of the HVA calcium current and consequently also the KC$_a$ current. Furthermore, the tendency for a rebound depolarization and firing caused by an LVA Ca$^{2+}$ current is also decreased. To examine the summed action of these mechanisms, we examined the effects of GABA$_B$ receptor activation with baclofen on NMDA-induced oscillations. These oscillations (Fig. 1) are characterized by four different phases (Brodin et al. 1991; Grillner and Wallén 1985; Sigvardt et al. 1985; Wallén and Grillner 1987): 1) an initial rapid depolarization (Fig. 1) caused by a regenerative opening of the voltage-dependent NMDA conductance, followed by 2) a plateau depolarization when the inward NMDA current is matched by an outward current through voltage-dependent K$^+$ channels. During the plateau a gradual Ca$^{2+}$ entry takes place, leading to the activation of KC$_a$ channels and a graded repolarization. At a certain potential the NMDA channels close 3), and a rapid repolarization (Fig. 1) follows. Thereafter, 4) a slow, gradual depolarization follows (Fig. 1) until the NMDA channels open again. Moreover, apamin, which blocks the KC$_a$ channels, markedly prolongs the duration of the plateau (El Manira et al. 1994), thus providing further support for the mechanisms described previously.

Membrane potentials were recorded intracellularly in the presence of 150 μM NMDA and 3 μM TTX (Fig. 2A). Addition of baclofen (40 μM, Fig. 2A) increased the cycle duration of the oscillations (Fig. 2B1, ---, $P < 0.001$). Because the shape of the oscillations is strongly voltage dependent, DCC techniques were used. Thus, by varying the amount of current injected continuously, the effects of baclofen can be compared over a range of trough potentials to be certain that the effects observed were not affected by changes in the trough potential. All cells (n = 8) were analyzed quantitatively with this paradigm. Figure 2 shows one example where an activation of GABA$_B$ receptors decreased the plateau duration (Fig. 2B2, ---, $P < 0.001$, n = 20) and increased the duration of the hyperpolarized phase (Fig. 2B3, ---, $P < 0.001$, n = 20) and the cycle duration (Fig. 2B1, ---, $P < 0.001$, n = 20). Baclofen decreased the plateau proportion (Fig. 2B4, ---, $P < 0.001$, n = 20), whereas the peak amplitude remained unchanged (Fig. 2B5, ---, 4/8 cells, $P > 0.1$, n = 20). The increase in the duration of the hyperpolarized phase and the decrease of the plateau proportion was observed in all cells (8/8 cells) and for all trough potentials investigated.

However, some variations were observed. In the cell shown in Fig. 3, double-peaked oscillations occurred in controls when the average trough potential was −72 mV (Fig. 3A1). Addition of baclofen (Fig. 3A2) increased the duration of the hyperpolarized phase (Fig. 3C2, ---, $P < 0.001$, n = 20). The cycle duration, however, remained unchanged at the −72-mV trough potential when double-peaked oscillations occurred (n = 3) (Fig. 3C1, indicated by *, $P > 0.1$, n = 20). Moreover, the plateau duration was also decreased by baclofen. Finally, the peak amplitude was reduced by GABA$_B$ receptor activation for all trough potentials (Fig. 3C3, ---, 4/8 cells, $P < 0.001$, n = 20).

To summarize, activation of GABA$_B$ receptors induces a shorter plateau and an increase of the hyperpolarized phase. The significance of this result is that it provides constraints on the interactive effects between the GABA$_B$ergic modulation of calcium and calcium-dependent potassium channels as detailed in the two following sections. First we address the role of LVA calcium channels in generating pacemaker oscillations, and in the following section the possibility that a KC$_a$ channel is activated by the LVA calcium is examined.

**Role(s) of LVA calcium channels during NMDA-induced, TTX-resistant membrane potential oscillations**

If the main effect of an activation of GABA$_B$ receptors is an opening of KC$_a$ channels an increase in both the duration of the
plateau and the hyperpolarized phases would be expected (El Manira et al. 1994; Tegnér et al. 1998). Baclofen, however, reduced the duration of the plateau phase, thus motivating an analysis of the influence of the LVA calcium component during pacemaker oscillations. To this end, a previously developed model of LVA calcium channels (Tegnér et al. 1997a) was used, which allows an independent analysis of the LVA component and the KCa channels, respectively.

When the LVA calcium current was added to the simulation model, NMDA-induced, TTX-resistant membrane potential oscillations still occurred (Fig. 4A1, solid line). The net LVA conductance, the \( m^3 h G_{\text{LVA}} \) factor (Fig. 4A2, solid line, see METHODS), is largest during the rising and initial part of the plateau phase and declines rapidly thereafter. LVA calcium channels become deinactivated during the hyperpolarized phase (increasing the value of the \( h \)-state variable, Fig. 4A3, solid line), thus allowing a non-0 current as the neuron depolarizes caused by rapid activation of the LVA calcium current (Fig. 4A4, solid line). The fast voltage-dependent inactivation of the LVA calcium current occurring during the plateau phase (Fig. 4A3, solid line) is responsible for the rapid decline of the LVA calcium conductance (Fig. 4A2, solid line).

A reduction of the LVA calcium current (80%, Fig. 4A1, dashed line) shortens the plateau phase and increases the hyperpolarized phase slightly (see Fig. 4A1), as indicated by the shaded scale bar that represents the duration of the hyperpolarized phase in control (solid line). The prolongation of the plateau phase by an LVA conductance is due to the addition of the net inward current carried by the LVA calcium (Fig. 4A2, solid line). The slight increase of the hyperpolarized phase when the LVA conductance is reduced is the result of two opposing effects. On the one hand a decreased net inward conductance (LVA) occurring during the rising phase would tend to increase the duration of the hyperpolarized phase. The duration of the hyperpolarized phase...
Polarized phase is, however, proportional to the level of calcium entering through NMDA channels, the Ca\textsubscript{NMDA} pool, which in turn activates a separate set of K\textsubscript{Ca} channels, referred to as K\textsubscript{CaNMDA} (Tegné 	extit{et al.} 1998). The level of calcium in the Ca\textsubscript{NMDA} pool, which decays slowly, builds up during the NMDA-induced oscillations, thus activating K\textsubscript{CaNMDA} channels, which have a hyperpolarizing influence during the hyperpolarized phase (Fig. 4A5, solid line). The peak level of the calcium in the Ca\textsubscript{NMDA} pool during the plateau phase is reduced when the LVA conductance is reduced (Fig. 4A5, dashed line). This reduction of the Ca\textsuperscript{2+} level tends to decrease the duration of the hyperpolarized phase. This was reproduced by simulating a reduced conductance of the K\textsubscript{CaNMDA} channels (10%, Fig. 4B, solid line) under control conditions to compensate for the increased Ca\textsuperscript{2+} inflow during the plateau phase and thus isolate the effect of removing the LVA conductance on the duration of the hyperpolarized phase. This permits a qualitative evaluation of the role of the LVA calcium channels during the hyperpolarized phase (Fig. 4B, solid line). A reduction of the LVA conductance under these conditions increases the duration of the hyperpolarized phase (compare Fig. 4B, dashed and solid lines; the shaded scale bar is of the same length as that in Fig. 4A).

To further elucidate the role of the LVA conductance on the duration of the hyperpolarized phase, the activation and deactivation curves of the LVA calcium current were shifted in the simulations between 1 and 5 mV (see Fig. 5 legend) in the hyperpolarizing direction. Because the LVA calcium current is modeled with an \( m^h \) form (Tegné 	extit{et al.} 1997a), this factor is calculated from the translated \( m \) and \( h \) components, as illustrated in Fig. 5A1. The duration of the plateau and hyperpolarizing phase of the corresponding \( m^h \) curves during NMDA-induced oscillations are shown in Fig. 5A2. Thus, as the “LVA calcium window” (the non-0 \( m^h \) factor) is shifted toward more negative potentials, the duration of the plateau phase and the hyperpolarized phase decreases (Fig. 5A2). Figure 5A3 shows that a reduction of an LVA calcium conductance has a clear effect on the duration of the hyperpolarized phase when the \( m^h \) factor is translated by -3 mV (compare with Fig. 4A1). The underlying mechanism is illustrated in Fig. 5B, in which the -5- and -5-mV example are compared (— and - - - - , respectively). The \( m^h \) factor is larger during the hyperpolarized phase as the \( m^h \) factor is shifted toward the hyperpolarized potential (see Fig. 5B, arrow). The level of calcium in the Ca\textsubscript{NMDA} pool is unchanged (compare Fig. 5B, — and - - - - ), thus ruling out that the effect on the hyperpolarized phase could be due to an effect on the K\textsubscript{CaNMDA} channels as in Fig. 4A.

In summary, a reduction of the conductance of the LVA calcium channel decreases the duration of the plateau phase, whereas the duration of the hyperpolarized phase is increased during the NMDA-induced oscillations. The increased duration of the hyperpolarized phase produced by GABA\textsubscript{B} receptor activation can be accounted for if the \( m^h \) curve is translated a few millivolts in the hyperpolarized direction (Fig. 5A1).

**Fig. 3.** Effects of baclofen on NMDA-induced, TTX-resistant membrane potential oscillations. A and B: intracellular recording from a spinal neuron with DCC. Bath application of 150 \( \mu \text{M} \) NMDA and 3 \( \mu \text{M} \) TTX induced membrane potential oscillations. GABA\textsubscript{B} receptors were activated by addition of 40 \( \mu \text{M} \) baclofen (A2). Average trough membrane potential was similar in both cases (-72.0 in A1 and -71.9 in A2, -76.5 in B1 and -76.2 in B2). Note the delayed depolarization in B2. The number of cycles in A1 is the same as that in B1. C, baclofen (- - - - ) increased the cycle duration (C1). *: presence of double-peaked oscillations (A1). Duration of the hyperpolarized phase (C2) was also increased. Peak amplitude was reduced in this cell by baclofen (4/8, see text). Average values (ordinate, 20 cycles) are plotted against the averaged trough potential (abscissa, mV, 20 cycles), which was modified by current injections, no larger than \( \pm 0.5 \text{ nA} \) in this neuron. Error bars are not plotted because they are in the limit of resolution of the data points.
NMBA-induced TTX resistant membrane potential oscillations (mV) with an LVA calcium conductance

A1

(●-●) G \(_{\text{Ca}}\) 80% reduced

A2

m \(^2\) h G \(_{\text{LVA}}\)

A3

Inactivation (h)

A4

Activation (m)

A5

Ca \(_{\text{NMDA}}\) pool

B

0.5 s

10% reduction of KCa activated by CaNMDA-pool in control, GLVA=1, (solid line)

Here we analyze whether an activation of a separate group of KCa channels (K\(_{\text{CaLVA}}\)) via LVA calcium channels is consistent with the effects of baclofen on the pacemaker oscillations. An LVA calcium pool (see METHODS and Tegnér et al. 1997a), which in turn activates a separate KCa conductance through a separate population of channels, is used. Figure 6 shows a parameter plot in which the following parameters are investigated independently of the level of LVA activation: 1) the strength of the coupling between the LVA calcium and the KCa channel interpreted either a) as the degree of activation of the KCa by the LVA calcium or b) simply the maximal conductance of the K\(_{\text{CaLVA}}\) channel (G\(_{\text{K(CaLVA)}}\)), which was actually the parameter modified during the simulations. The contribution of the LVA calcium channel to the total KCa current is given by Eq. 5 (METHODS).

The second parameter analyzed was 2) the decay time of the calcium in the intracellular calcium pool and finally 3) the NMDA levels [1.0, 2.0 arbitrary units (A. U.)]. A sample trace of the stimulated NMDA-induced oscillations during 2 s is shown in each box (Fig. 6). The kinetics of the decay of the intracellular calcium in the LVA pool (\(\bar{G}_{\text{LVA}}\), see Eq. 2) is referred to as slow (2 s\(^{-1}\)), medium (10 s\(^{-1}\)), or fast (20 s\(^{-1}\)). The numbers indicate the different values of the parameter for the decay of the intracellular calcium in the LVA pool. For example, in the Fig. 6, topmost left box, the following parameter values apply: NMDA level is 1.0 (A. U.), kinetics of the decay of the intracellular calcium is 2 (s\(^{-1}\)), and the conductance of the K\(_{\text{CaLVA}}\) is 0; thus \(G = 0\). This example will be referred to as [KCa(slow), G(0), N(1.0)]. By using this notation we make the following observations (Fig. 6). 1) Addition of a \(G_{\text{K(CaLVA)}}\) conductance from 0 to 0.8 shortened the duration of the plateau phase irrespective of the kinetics of the intracellular calcium pool and the NMDA level. For example, compare the records in the left and right columns of Fig. 6 at different NMDA levels. The K\(_{\text{CaLVA}}\) conductance thus acts as a plateau terminating factor because, for a given level of intracellular calcium, the influence of a K\(_{\text{Ca}}\) channel became stronger when the \(G_{\text{K(CaLVA)}}\) conductance was increased. Moreover, 2) with a slow decay of the intracellular calcium, the duration of the hyperpolarized phase increases as the \(G_{\text{K(CaLVA)}}\) conductance increases (compare [KCa(slow), G(0.2), N(1.0)] with [KCa(slow), G(0), N(1.0)]). Thus, although the duration of the plateau is decreased, the effective inhibition during the hyperpolarized phase is increased because of the slow decay rate of the intracellular LVA calcium. 3) If instead the decay rate of the intracellular calcium is increased to a medium or fast rate, the duration of the hyperpolarized phase may instead decrease by an addition of a \(G_{\text{K(CaLVA)}}\) conductance (compare left and right columns, Fig. 6). As shown by comparing the middle and right columns a decrease in the duration of the hyperpolarized phase is obtained with an increased conductance of the \(G_{\text{K(CaLVA)}}\). Note also 4) that an increased conductance of the \(G_{\text{K(CaLVA)}}\) reduces the peak depolarization (compare middle and right column, Fig. 6). Finally, 5) the effects of increasing the K\(_{\text{CaLVA}}\) are more clear at a lower level of NMDA because the level of NMDA had a weaker depolarizing influence compared with the higher levels of NMDA.

Activation of GABA\(_A\) receptors increases the duration of the hyperpolarized phase (Figs. 2B3, - - - - , and 3C3, - - - - ), whereas a decreased conductance of the K\(_{\text{CaLVA}}\) channels would decrease the duration of the hyperpolarized phase if the kinetics are slow (compare [KCa(slow), G(0.8), N(2.0)] with [KCa(slow), G(0.2), N(2.0)] and [KCa(slow), N(2.0)] in Fig. 6). Thus it is not consistent with the experimental

Simulation of KCa channels activated by the calcium entering through LVA calcium channels

Here we analyze whether an activation of a separate group of KCa channels (K\(_{\text{CaLVA}}\)) via LVA calcium channels is consistent with the effects of baclofen on the pacemaker oscillations. An
data to assume that the kinetics of the intracellular LVA calcium pool is of the slow type if the LVA calcium activates a KCaLVA channel. The simulations suggest a weaker coupling between the LVA calcium and the corresponding KCaLVA channel (or smaller maximal conductance of KCaLVA) compared with the KCaAP and KCaNMDA channels. The effect of a modulation of the G_K(CaLVA) conductance would be too marked if, for example, G = 0.8 is taken as a reference value (see DISCUSSION) because of the finding that GABA_B receptor activation decreases the duration of the plateau phase (Figs. 2B2, ---, and 3C2, ----). In conclusion, fast kinetics and low conductance of the putative KCaLVA channel is consistent with the experimental data obtained on the pacemaker oscillations.

Modulation of firing properties in the absence of pacemaker oscillations

It is not clear what overall effect GABAergic modulation will have on neuronal firing if GABA receptor activation reduces both LVA and HVA calcium currents and KCa currents. Here we examine the influence of GABAergic modu-

![Diagram of G_K(CaLVA) activated by the CaLVA-pool](image)

**FIG. 6.** Computer simulations of the contribution of a KCa conductance, activated by calcium entering through LVA channels, to the NMDA-induced, TTX-resistant membrane potential oscillations. The following parameters were analyzed (see text): decay time of the LVA calcium (left), the level of NMDA (right), and the maximal conductance of the KCa channel activated by the LVA calcium (G_K(CaLVA), top).
only suppressed the $K_{CaAP}$ channels. It was sufficient to include a $K_{CaLVA}$ channel with a low conductance, as determined earlier (Fig. 6), which became activated by the LVA calcium during the subthreshold current pulse (Fig. 7B1). The simulated reduction of the $K_{CaLVA}$ (and $K_{CaAP}$) channels by apamin induced a spike (Fig. 7B2), thus reproducing the earlier experimental result (El Manira et al. 1994).

Apolmin-sensitive $K_{CaAP}$ channels are the main determinant of the spike frequency regulation in lamprey neurons (Hill et al. 1992; Meer and Buchanan 1992). It is less clear to what extent LVA calcium channels contribute to the firing properties of these neurons. In the simulations described subsequently we studied the influence of the LVA calcium itself without an accompanying $K_{CaLVA}$ channel to identify a possible role of LVA calcium channels per se. Figure 8A1 shows the firing response to a current pulse for a model neuron without an LVA calcium channel. The first spike interval (Fig. 8A1) is shorter than the second and third interval in controls, which is similar to experimental results (Buchanan 1993; Wallén et al. 1989). If an LVA conductance is added to the model neuron (Fig. 8B1), everything else being equal, the first interspike interval became shorter, as did the following interval, albeit to a lesser extent. As the neuron is depolarized, the LVA calcium conductance rapidly activates, whereas it inactivates during the plateau phase on which the spikes are fired. Thus the $m'h$ factor rises initially but declines during the spike train (Fig. 8B2). A simulated reduction of the $K_{CaAP}$ channel increases the number of spikes and decreases the first spike interval (Fig. 8A2) in agreement with earlier experiments (El Manira et al. 1994; Meer and Buchanan 1992). The simulations suggest that, if the main action of baclofen were mediated via LVA calcium channels (Figs. 1–4), the number of spikes would tend to decrease, and the first spike interval would increase if GABA$_B$ receptors were activated. On the other hand, if the indirect effects on the $K_{CaAP}$ channels were dominant, an increased number of spikes would be expected. To further examine this, spinal neurons were stimulated by square-wave current pulses (Fig. 9). In all cells ($n = 5$) recorded we observed the following. 1) The number of spikes was reversibly reduced when

- **Figure 7**: Role of LVA calcium on firing properties around threshold simulations. A: current pulse ($200 \text{ ms} \times 0.34 \text{ nA}$) induced a spike in control (A1) but not when the LVA conductance was removed (A2). B: 100-ms current pulse was given to a model neuron (B1) equipped with 2 types of $K_{Ca}$ channels ($K_{CaAP}$ and $K_{CaLVA}$). These were turned off in B2, simulating the effect of apamin on a neuron with an LVA calcium conductance. The double lines in A1 and B2 indicate that the spikes were truncated.

- **Figure 8**: Role of LVA calcium on the firing properties above threshold simulations. A: 100-ms current pulse ($1.3 \text{ nA}$) was given to a model neuron with no LVA properties in control (A1). Note the decreased duration of the first spike interval as the $K_{CaAP}$ conductance is turned off in A2. B: similar to A1 except that an LVA conductance was added to the model neuron. Note the decreased duration of the first spike interval as indicated by ---, which equals the duration of the first spike interval in A1. In B2 the net $m'h$ factor was calculated during the spike train in B1. The decreased amplitude is due to progressive inactivation of the LVA conductance during the pulse.
baclofen was added (compare Fig. 9, A1 and A2), and 2) a significant ($P < 0.0001$) increase in the duration of the first spike interval occurred (Fig. 9, A1, A2, and B). Thus the effects of baclofen on the spikes (Fig. 9) correspond well to a simulated reduction of the LVA conductance (Fig. 8).

In summary, both the simulations and the experiments on the GABA$_B$ergic modulation of the firing properties in the absence of pacemaker oscillations support the notion that the primary mechanism accounting for the effects of baclofen is the calcium entry through LVA channels.

**DISCUSSION**

The objective of this study was to clarify the relative contribution of LVA calcium channels and $K_{Ca}$ channels in the modulatory actions of GABA$_B$ receptors. To this end we used the NMDA-induced oscillations as an experimental and computational model to study the interactive effects between LVA and HVA calcium channels and $K_{Ca}$ channels activated by either LVA or HVA calcium channels. We have shown that the direct effect on the LVA calcium channels could account for the modulatory effects of baclofen on the NMDA-induced oscillations. The indirect effect on the $K_{Ca,AP}$ channels via HVA calcium channels could not account for the modulation of the depolarized and hyperpolarized phase by baclofen. Both simulations and experiments revealed that the LVA calcium conductance could contribute to the duration of the first spike interval.

**Role of calcium and calcium-dependent conductances during NMDA-induced oscillations**

The importance of the NMDA-induced oscillations in this context is essentially twofold. 1) It provides an experimental/computational model allowing an analysis of the interactions between various intrinsic membrane properties and 2) a source for intrinsic oscillations that could be of importance in the generation of rhythmic patterns underlying locomotion in the lamprey spinal cord. Thus, to understand the mechanisms underlying the generation of locomotion at the cellular level, it is important to understand how the different conductances interact to produce membrane potential oscillations. We focused on the cellular interplay between calcium conductances and their respective $K_{Ca}$ channels without considering their role in the operation of the neural network. Earlier studies have shown that both mechanisms can modulate the burst rate in the spinal segmental network (El Manira et al. 1994; Tegnér et al. 1997a, 1998).

Figure 10 summarizes the influences of calcium channels (HVA and LVA type) and $K_{Ca}$ conductances with either fast or slow intracellular calcium dynamics. It shows their influence on the duration of the plateau and the hyperpolarized phase.
The duration of the plateau phase is determined by a balance between Ca$^{2+}$ channels of either the HVA or the LVA type, which tend to increase the duration, and a KCa conductance, which decreases the duration of the plateau phase irrespective of the dynamics of the intracellular calcium activating the KCa channel. The duration of the hyperpolarized phase is decreased by the addition of an LVA calcium channel to the model neuron (Figs. 4 and 5). This effect becomes more dramatic the larger the $m^h$ factor is at the potential at which the transition between hyperpolarization and depolarization occurs (see Fig. 5A1, left - - - -). Moreover, the introduction of a KCa channel with slow dynamics, such as the KCaNMDA channel (Brodin et al. 1991; Ekeberg et al. 1991), increases the duration of the hyperpolarized phase. Thus a given level of NMDA has a depolarizing influence, which activates the KCaNMDA channels in proportion to the calcium entering via the NMDA channels. These factors together determine the depolarized and hyperpolarized phase in a voltage-dependent manner because of the voltage dependence of the NMDA receptor. The modulatory action of the HVA, LVA, KCaAP, and KCaLVA channels further contributes to the regulation of the depolarized and hyperpolarized phases as described previously. It is now important to further characterize different subtypes of HVA Ca$^{2+}$ channels (El Manira and Bussières 1997) and investigate their influence on NMDA-induced oscillations experimentally.

**LVA calcium and the KCaAP contribution to the modulatory effects of GABA$_B$ receptor activation**

Transmitters act as a rule via several subtypes of receptors (Nicoll 1988). Moreover, a given receptor subtype may mediate its effects through one of a wide variety of mechanisms. It is therefore important to investigate the relative contribution of the different components in the effects mediated by a given transmitter. Activation of GABA$_B$ receptors in the lamprey reduces the LVA and HVA calcium current, which in turn reduces the peak amplitude of the slow AHP (Matsushima et al. 1993). Thus the arguments that indicate a predominant influence of the LVA calcium channels compared with the indirect effect on the KCaAP channels in lamprey are as follows. 

1) During the NMDA-induced oscillations baclofen decreases the duration of the plateau phase, whereas the duration of the hyperpolarized phase is increased (Figs. 2 and 3). 2) A simulated reduction of the LVA calcium conductance can account for the effects of baclofen during the NMDA-induced oscillations (Figs. 4 and 5), and 3) apamin (Blatz and Magleby 1986), which directly depresses the KCa current, increases both the duration of the plateau and the hyperpolarized phase in a voltage-dependent manner because of the voltage dependence of the NMDA receptor. The modulatory effects of GABA$_B$ receptors on NMDA-induced oscillations, however, provide additional experimental constraints on the effects of the LVA calcium. We used these data to assess whether a KCaLVA channel was activated and to determine the basic characteristics of the calcium kinetics involved. We found that it was not consistent with experimental data on NMDA-induced oscillations (Figs. 2 and 3) to have slow intracellular calcium kinetics for the LVA calcium. Moreover, the maximal conductance of the KCaLVA appears to be small compared with the conductance of KCaAP and KCaNMDA (Fig. 6). Thus fast decay kinetics of the intracellular LVA Ca$^{2+}$ together with a small conductance of the KCaLVA is consistent with the experiments on NMDA-induced oscillations. However, these conclusions are based on experiments with the modulatory effects of baclofen on the slow NMDA-induced oscillations.

Recent studies (McCobb and Beam 1991; Olsen and Calabrese 1996) have shown that the amount and characteristics of LVA and HVA calcium currents also depend on the envelope of the voltage curve. In a model of leech heart interneurons it was predicted that LVA calcium currents were partially inactivated during a slow voltage ramp to a plateau potential (Nadim et al. 1995; Olsen et al. 1995). A similar result (a reduced $m^h$ factor) was obtained when the effects of GABA$_B$ receptors were simulated with a sinusoidal current stimulation accounting for the effects of LVA calcium channels at the single cell and network level described by Tegnér et al. (1997a), it was not necessary to assume that the LVA calcium activated a KCa channel.

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**LVA, KCaLVA channels, and interactive effects contributing to firing properties**

It is often difficult to characterize the kinetics and conductance of a KCa channel because the level of intracellular Ca$^{2+}$, the contribution of Ca$^{2+}$ from various Ca$^{2+}$ channels, and the intracellular kinetics are generally not known in sufficient quantitative detail. A simplifying step is to lump the open-closed transitions of channels and intracellular transition schemes into differential equations (Ekeberg et al. 1991). In accounting for the effects of LVA calcium channels at the single cell and network level described by Tegnér et al. (1997a), it was not necessary to assume that the LVA calcium activated a KCa channel.

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Potential is hyperpolarized, and the duration of the first spike interval is the shortest, the firing increases as the holding channels, and here it has also been shown that the first spike sgaard 1996a). This is due to the presence of LVA calcium channels. It has also been shown that the first spike interval decreases accordingly. Moreover, in another group of neurons that generate plateau potentials (Russo and Hounsgaard 1996b), apamin increases the firing rate and decreased the duration of the first spike interval, a finding that also applies to motorneurons (Hounsgaard et al. 1988). Finally, a blockade of the L-type calcium channel decreased the firing rate and increased the duration of the first spike interval compared with the spike train elicited when apamin was applied (Hounsgaard and Mintz 1988). Thus the inward calcium current itself influences the first spike interval in the turtle spinal cord, similar to the role of LVA calcium channels suggested in this study. Furthermore, both in the turtle and lamprey spinal cord neurons, the presence of an inward calcium current affects the number of spikes elicited. In conclusion, the results on spinal neurons in the turtle are qualitatively similar to the firing properties of lamprey neurons presented here (Fig. 7–9) and in earlier studies (Buchanan 1993; El Manira et al. 1994; Meeder and Buchanan 1992).

This study shows that a modulation of the LVA calcium current can account for the effects of baclofen on NMDA-induced oscillations and firing properties of lamprey spinal neurons. Furthermore, the experiments and simulations taken together suggest that a low-conductance K_{Ca,LVA} channel of the apamin-sensitive type could be activated by the calcium (with a fast decay) entering through LVA calcium channels.

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