Modulation by Extracellular pH of Low- and High-Voltage-Activated Calcium Currents of Rat Thalamic Relay Neurons

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Shah, Mukesch Johannes, Susanne Meis, Thomas Munsch, and Hans-Christian Pape. Modulation by extracellular pH of low- and high-voltage-activated calcium currents of rat thalamic relay neurons. J Neurophysiol 85: 1051–1058, 2001. The effects of changes in the extracellular pH (pH\textsubscript{o}) on low-voltage- (LVA) and high-voltage- (HVA) activated calcium currents of acutely isolated relay neurons of the ventrobasal thalamic complex (VB) were examined using the whole cell patch-clamp technique. Modest extracellular alkalinization (pH 7.3 to 7.7) reversibly enlarged LVA calcium currents by 18.6 ± 3.2% (mean ± SE, n = 6), whereas extracellular acidification (pH 7.3 to 6.9) decreased the current by 24.8 ± 3.1% (n = 9). Normalized current amplitudes (I/I\textsubscript{h}) fitted as a function of pH\textsubscript{o} revealed an apparent pK\textsubscript{a} of 6.9. Both, half-maximal activation voltage and steady-state inactivation were significantly shifted to more negative voltages by 2–3 mV on extracellular acidification, respectively. Recovery from inactivation of LVA calcium currents was not significantly affected by changes in pH\textsubscript{o}. In contrast, HVA calcium currents were less sensitive to changes in pH\textsubscript{o}. Although extracellular alkalinization increased maximal HVA current by 6.0 ± 2.0% (n = 7) and extracellular acidification decreased it by 11.9 ± 0.02% (n = 11), both activation and steady-state inactivation were only marginally affected by the moderate changes in pH\textsubscript{o} used in the present study. The results show that calcium currents of thalamic relay neurons exhibit different pH\textsubscript{o} sensitivity. Therefore activity-related extracellular pH transients might selectively modulate certain aspects of the electrogenic behavior of thalamic relay neurons.

INTRODUCTION

Both normal and pathological neuronal activity is accompanied by distinct changes in extracellular and/or intracellular pH (for review see Chesler and Kaila 1992; Kaila and Ransom 1998). Even small changes in pH\textsubscript{i} and/or pH\textsubscript{o} have been shown to affect normal synaptic transmission and a variety of ligand-gated and voltage-gated ion channels, as, for instance, glutamate and GABA receptors (Pasternack et al. 1992; Traynelis 1998) and voltage-dependent calcium channels (Church 1999; Kiss and Korn 1999; Mironov and Lux 1991; Tombaugh and Somjen 1996, 1997). Alkaline pH\textsubscript{o} has been shown to increase calcium currents, whereas acidic pH\textsubscript{o} decreases the current (Tombaugh and Somjen 1997). Alkaline pH\textsubscript{o} has been shown to increase calcium currents, whereas acidic pH\textsubscript{o} decreases the current (Tombaugh and Somjen 1997). Moreover, low-voltage- (LVA) and high-voltage- (HVA) activated calcium currents seem to exhibit differential sensitivity to changes in pH\textsubscript{i} (Tombaugh and Somjen 1997). Alkaline pH\textsubscript{i} has been shown to increase calcium currents, whereas acidic pH\textsubscript{i} decreases the current (Tombaugh and Somjen 1998). However, it remains unclear to what extent changes in pH\textsubscript{i} might differentially affect either LVA or HVA calcium currents.

Thalamic relay neurons possess both HVA and LVA calcium currents (Hernandez-Cruz and Pape 1989), which critically determine their firing pattern. HVA calcium currents in thalamic relay neurons are thought to regulate tonic firing of action potentials at depolarized membrane potentials (Budde et al. 2000; Guyon and Leresche 1995; Kammermeier and Jones 1997; Zhou et al. 1997). The pH sensitivity of HVA calcium currents has been studied in detail in other types of neurons (Mironov and Lux 1991; Tombaugh and Somjen 1996, 1997; Zhou and Jones 1996).

The LVA calcium current, by comparison, is considered an important element in the generation of rhythmic oscillatory electrical activity, in that it underlies the generation of a low-threshold calcium spike (LTS) (Llinas and Jahnsen 1982; McCormick and Bal 1997; Steriade et al. 1993). Oscillatory generation of LTSs is controlled by the interplay of LVA calcium current and a hyperpolarization-activated cation current (I\textsubscript{h}), and synaptic mechanisms (McCormick and Bal 1997). Recently we have shown that voltage dependence of activation of I\textsubscript{h} is influenced by pH\textsubscript{i} rather than pH\textsubscript{o} (Munsch and Pape 1999). In comparison, pH-modulation of LVA calcium currents in neurons is less well understood.

We have therefore addressed the question of the pH\textsubscript{i} sensitivity of LVA and HVA calcium currents in thalamic relay neurons and the consequences of a modulation of LVA currents by extracellular protons for LTS generation by the use of patch-clamp techniques on thalamic relay neurons either acutely isolated from the rat ventrobasal thalamic complex (VB) or within an in situ slice preparation of VB.

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METHODS

Preparation

Long Evans rats of either sex [postnatal day 11–17 (P11–P17)] were anesthetized with halothane and decapitated. A block of tissue containing the VB was quickly removed from the rest of the brain and placed in chilled oxygenated PIPES-buffered solution containing (in mM) 123 NaCl, 2.4 KCl, 10 MgSO\(_4\), 0.5 CaCl\(_2\), 20 PIPES, 23 sucrose, and 10 dextrose; pH 7.25. Coronal slices (300–400 \(\mu\)m) containing the VB were made from the thalamus. For in situ whole cell patch-clamp measurements, thalamic slices were immediately transferred to a submersion chamber filled with artificial cerebrospinal fluid (ACSF) containing (in mM) 120 NaCl, 2.5 KCl, 22–26 NaHCO\(_3\), 1.25 NaHPO\(_4\), 2 MgSO\(_4\), 2 CaCl\(_2\), and 10 glucose. pH was adjusted to 7.35 with 95% O\(_2\)-5% CO\(_2\). Slices were maintained at room temperature (22–25°C).

For acute isolation of neurons, the VB was carefully dissected from neighboring tissue under stereoscopic observation. Slices were placed in an oxygenated PIPES solution containing (in mM) 20 PIPES, 115 NaCl, 5 KCl, 2.6 MgCl\(_2\), 25 dextrose, and 0.5 CaCl\(_2\), pH 7.35 and warmed up to 30°C. Slices were then incubated in protease-containing (1 mg/ml) PIPES solution for 25–40 min.

After washing in enzyme-free medium, the neurons were mechanically dissociated by trituration with fire-polished Pasteur pipettes.

Electrophysiological recording

For in situ recordings, whole slices were transferred to an experimental chamber. Individual cells were approached by visual control with differential interference contrast infrared (DIC-IR) videomicroscopy. Recordings were made with an EPC-9 amplifier operating Pulse software (HEKA, Lambrecht, Germany). Measurements were performed at room temperature (22–25°C) in ACSF. The composition of the pipette solution used for current-clamp experiments was as follows (in mM): 95 K-gluconate, 20 K-citrate, 10 NaCl, 10 HEPES, 1 MgCl\(_2\), 0.5 CaCl\(_2\), 1 K-bis-(o-aminophenoxy)-N,N,N',N'-tetraacetic acid (BAPTA), 3 Mg-ATP, and 0.5 Na\(_2\)-GTP, pH adjusted to 7.4 with KOH. The pH of HCO\(_3\)-buffered solutions at constant pCO\(_2\) (5%) was changed by substitution of NaCl equimolarly by different amounts of [HCO\(_3\)]\(^{-}\); 7–9 mM for pH 6.9, 18–20 mM for pH 7.3, and 45–47 mM for pH 7.7. Actual bath pH was checked with a pH electrode and adjusted by adding small amounts of NaHCO\(_3\) to the stock solution.

Acutely isolated cells were electrophysiologically analyzed with the patch-clamp technique in whole cell voltage-clamp mode using an EPC-7 amplifier (List Medical System, Darmstadt, Germany). Patch pipettes were made from borosilicate glass (GC150TF-10, Clark Electromedical Instruments, Pangbourne, UK). Typical electrode resistance was 2–5 MΩ, with access resistance of 6.75 ± 0.07 MΩ (mean ± SE, \(n = 68\)). Records were low-pass filtered at 2.5 kHz (8-pole Bessel filter). Voltage-clamp experiments were performed using PClamp software operating via Labmaster interface (Axon Instruments, Foster City, CA) on an IBM computer. Each test pulse at various \(\phi H\) values was preceded by a prepulse to −110 mV to regard possible pH effects on leakage currents. However, there was no significant difference between leakage currents at the \(\phi H\) values used. Leakage currents were then compensated off-line by estimating the electronic, ohmic component of the membrane current during the prepulses and subtracting each of the scaled values from the corresponding current traces.

Isolated neurons were continuously superfused (0.1–1 ml per minute) with extracellular solution containing (in mM) 134 NaCl, 10 HEPES, 2 KCl, 15 dextrose, 15 mannitol, 2 CaCl\(_2\), 3 MgCl\(_2\) (pH 7.34 with NaOH). A multibarreled laminar-flow perfusion system (0.1 ml/min) was placed close to the recorded neuron allowing to completely change the solution surrounding the recorded cell within <500 ms. For analyzing the current-voltage (I-V) relationship, kinetics, activation, and inactivation at \(\phi H\) 6.9, 7.3, 7.7, and 8.1 we used a HEPES-buffered solution containing (in mM) 120 NaCl, 1 KCl, 3 CaCl\(_2\), 1 MgCl\(_2\), 20 dextrose, 10 mannitol, 6 4-aminopyridine, 20 TEA, 0.0015 TTX, and 10 HEPES. pH was adjusted to the desired value with NaOH or HCl. For all acidic solutions below a \(\phi H\) of 6.9, HEPES was replaced with equimolar PIPES.

The internal solution contained 70 mM CsCl, 15 mM CsOH, 10 mM NaCl, 1 mM KCl, 11 mM EGTA, 2 mM MgCl\(_2\), 1 mM CaCl\(_2\), 20 mM TEA, 5 mM ATP, 0.5 mM GTP, 15 mM phosphocreatin, 50 units creatine kinase, and 50 mM HEPES to minimize changes of internal pH (Irisawa and Sato 1986). The internal solution was adjusted to \(\phi H\) 7.2 with CsOH. Experiments were performed at room temperature (21–24°C). After establishing the whole cell configuration, active currents were monitored by periodic voltage steps until the current had stabilized. Recordings began typically 3–6 min after rupturing the membrane patch.

Isolation of LVA and HVA currents

LVA and HVA calcium components were separated using a conditioning pulse protocol (Tsakiridou et al. 1995). A 100-ms prepulse to −50 mV between the hyperpolarizing pulse to −110 mV (1,000 ms) and the depolarizing voltage steps was used to inactivate LVA calcium currents. LVA calcium currents were isolated by digital subtraction of records obtained with and without the conditioning prepulse. Pharmacological tools to separate different calcium currents were renounced, because of possible \(\phi H\)-dependent effects of the drugs (Galizi et al. 1984; Platt et al. 1993).

Data analysis

Analyses of current records, including curve fitting, were done with the use of PClamp software. Inactivation and activation curves were obtained by fitting the data points to a Boltzmann function \(y = A2 + (A1 – A2)/(1 + exp(x – x0/dx))\), where \(x0\) is the half-maximal activation voltage, \(dx\) is the slope factor, and \(A1\) and \(A2\) are constants. Data are presented as means ± SE and were statistically evaluated using a paired t-test (Origin 4.1).

RESULTS

\(\phi H\) effects on LVA calcium currents

LVA calcium currents could be evoked by depolarizing voltage steps from a prepotential of −110 mV to various test potentials in acutely isolated VB relay neurons (Fig. 1, A and C). Currents activated at −70 mV and peaked near −40 mV. Modest changes in the \(\phi H\) of the superfusing solution led to distinct shifts in the peak amplitude of the I-V curve. Extracellular acidification from \(\phi H\) 7.3 to 6.9 caused a decrease of the peak amplitude of LVA calcium currents of 24.8 ± 3.1% (\(n = 9\)), whereas extracellular alkalization reversibly increased peak currents by 18.6 ± 3.2% (\(n = 6\); Fig. 1, B and D). By fitting normalized current amplitudes (\(I/\phi H_{50}\)) as a function of the \(\phi H\) of the extracellular solution (\(\phi H_{exo}\)), a Hill-plot was obtained, from which an apparent \(pK_a\) of 6.9 was estimated (slope factor = 14.5; Fig. 1E). Peaks of the I-V curves were not significantly shifted along the voltage axis by either acidic \(\phi H_{exo}\) (6.9) or alkaline \(\phi H_{exo}\) (7.7; Fig. 1, B and D).

To determine the effects of \(\phi H_{exo}\) on gating properties of LVA calcium channels, activation curves were obtained from tail currents elicited by stepping to −80 mV from various depolarizing test potentials as shown in inset of Fig. 2A. Activation...
curves were reversibly shifted by changes in pH\textsubscript{o}. Acidic pH\textsubscript{o} (6.9) moved the half-maximal activation voltage from $-54.9 \pm 1.9$ mV ($n = 4$) to $-51.6 \pm 1.3$ mV ($n = 4$; Fig. 2A). Conversely, alkaline pH\textsubscript{o} (7.7) significantly ($P \leq 0.05$) shifted the half-maximal activation voltage to more negative potentials from $-53.8 \pm 1.2$ mV ($n = 5$) to $-55.8 \pm 1.1$ mV ($n = 5$; Fig. 2B). Time for current activation showed typical voltage dependence with time constants ($\tau$) becoming larger at more negative voltages (Fig. 2, C and D). Time constants were not significantly affected by changes in pH\textsubscript{o} at potentials more positive than $-30$ mV but increased at more negative potentials for acidic pH\textsubscript{o} (Fig. 2C) and decreased for alkaline pH\textsubscript{o} (Fig. 2D), respectively.

Steady-state inactivation of LVA calcium current was analyzed with conditioning pulses of 1,000 ms duration between $-110$ and $-45$ mV followed by a constant test pulse to $-40$ mV (inset of Fig. 3A). Inactivation curves were obtained from plots of the normalized current ($I/I_{\text{max}}$) versus the potential of the conditioning prepulse. The inactivation curves were fitted with a Boltzmann equation. Changing the external pH from 7.3 to 6.9 caused a significant ($P \leq 0.05$) shift of the half-maximal voltage ($V_{1/2}$) of $1.85 \pm 0.6$ mV ($n = 7$) to more positive potentials (Fig. 3A). Conversely, alkaline pH\textsubscript{o} (7.7) reversibly shifted $V_{1/2}$ by $2.6 \pm 0.7$ mV ($n = 7$) to more negative potentials without changing slope parameters (Fig. 3B). Time constants of inactivation were determined by fitting single exponential equations to the decay of individual LVA calcium current traces. The rate of current inactivation became faster at more depolarized potentials (Fig. 3, C and D). Again, time constants of inactivation were unaffected by pH\textsubscript{o}, at $-30$ mV or more positive potentials, but differed significantly at more negative potentials. For instance, at $-50$ mV, extracellular acidification resulted in a slower rate of inactivation and, conversely, alkaline pH\textsubscript{o} led to a faster inactivation of LVA calcium currents (Fig. 3, C and D).

To study the time course of recovery from inactivation, cells...
were held at a potential of −50 mV to inactivate LVA calcium currents completely. Hyperpolarizing command steps to −110 mV were then applied with increasing intervals followed by a constant test pulse to −40 mV. Increasing the duration of hyperpolarization was accompanied by an increase in current amplitudes. The fractional recovery of normalized currents (I/I_{max}) showed the typical time dependence (data not shown). A significant pH_{o} dependence was not observed.

FIG. 2. pH_{o} dependence of LVA calcium current activation. A and B: activation curves of LVA calcium currents before, during, and after exposure of cells to either acidic (pH_{o} = 6.9, n = 4) or alkaline (pH_{o} = 7.7, n = 5) solution. Data points represent means of normalized current amplitudes (I/I_{max}). Error bars were omitted for clarity. Continuous lines represent best fits of a Boltzmann distribution to the data points. Insets: voltage protocol (A) and sample current traces (B). Extracellular acidification shifted the activation curve to more positive voltages, whereas extracellular alkalinization shifted activation to more negative voltages. C and D: plots of time constants (τ) of current activation as a function of membrane voltage (V_m). Data represent means ± SE. Values significantly (P ≤ 0.05) different from control are marked by asterisks.

FIG. 3. pH_{o} dependence of LVA calcium current inactivation. A and B: inactivation curves of LVA calcium currents obtained from plots of the normalized current (I/I_{max}) at −40 mV vs. the potential of the conditioning prepulse under control conditions and during exposure to pH_{o} 6.9 (n = 7, A) and 7.7 (n = 7, B), respectively. Data represent mean values. Error bars were omitted for clarity. Continuous lines represent Boltzmann fits to data points. Insets: voltage protocol (A) and sample current traces (B). Steady-state inactivation was shifted to more positive voltages at acidic pH_{o} and to more negative voltages at alkaline pH_{o}, respectively. C and D: time constants (τ) for current inactivation at different pH_{o} values plotted as a function of membrane voltage (V_m). Data points represent means ± SE. Values significantly (P ≤ 0.05) different from control are marked by asterisks.
**pH effects on HVA calcium currents**

HVA calcium currents were isolated through inactivation of the LVA currents by a 100 ms prepulse to −50 mV (Kammermeier and Jones 1997). Sustained inward currents could be activated by step depolarizations above −40 mV (Fig. 4, A and B), and the I-V curve revealed a peak near −5 mV (Fig. 4, C and D). At acidic pH₀ (6.9), HVA calcium currents became depressed by 11.9 ± 2.6% (n = 11), while current amplitudes were increased at alkaline pH₀ (7.7) by 6.0 ± 2.0% (n = 7; Fig. 4, C and D). A Hill-fit of normalized peak HVA calcium currents (I/Iₘ₅ₑₓₜ) plotted as a function of pH₀ revealed an apparent pKₐ of 6.5. The moderate changes in pH₀ used in the present experiments only marginally affected the half-maximal activation and the rate of HVA calcium current activation (data not shown). Also, neither moderate extracellular acidosis (6.9, n = 5) nor alkalosis (7.7, n = 6) altered the steady-state inactivation (P ≥ 0.3). The rate of HVA calcium current inactivation was only marginally affected by either acidic pH₀ (6.9, n = 11) or alkaline pH₀ (7.7, n = 7), but this effect was more variable and seldom reached statistical significance (data not shown). The small effects of pH₀ changes on HVA channel gating are likely due to differential pH₀ sensitivity of HVA subtypes, but this was not further investigated.

**Effects of bath pH on burst activity**

The consequences of pH₀ effects on calcium currents for the electrogenic activity of relay neurons were tested in an in vitro slice preparation of the thalamus containing the VB. We concentrated on low-threshold burst activity in view of the well-documented contribution of the LVA current to this type of activity. By repetitive hyperpolarization through injection of negative current pulses, rebound LTSs can be evoked, which mimics rhythmic oscillatory activity as occurs during burst mode, the major activity pattern of relay neurons at relatively hyperpolarized membrane potentials (McCormick and Bal 1997). In a brain slice superfused with a bath solution of pH 7.3 (control, HEPES-buffered) repetitive injection of hyperpolarizing current (15 × 100 ms pulses at a frequency of 2.5 Hz) under current-clamp conditions was adjusted to elicit LTSs at every second current pulse (Fig. 5A, top trace, n = 6). These LTSs were typically crowned by 2–5 fast sodium/potassium action potentials (inset of Fig. 5, A and B). Changing bath pH to 6.9 significantly reduced the frequency of LTS generation, whereas a more alkaline bath pH (7.7) caused a significant increase in LTS frequency (Fig. 5A, middle and bottom traces). The same results were obtained for brain slices bathed in solutions with HCO₃⁻ as the main pH buffer (Fig. 5B). These results are summarized in Fig. 5, B and D. Acidic pH₀ (n = 6) significantly decreased the frequency of LTS generation (quantified as number of Ca²⁺ bursts/current pulse train), whereas alkaline pH₀ (n = 6) increased LTS frequency (Fig. 5, C and D).

**DISCUSSION**

The present investigation indicates that extracellular H⁺ may act as a modulator of LVA calcium currents due to proton-induced changes in the activation properties of the underlying channels, thereby relating activity-dependent extracellular pH transients to changes in the activity patterns of thalamic relay neurons.

**pH₀ versus pHᵢ effects on calcium currents**

Changes in pH₀ have been shown to affect intracellular pH in smooth muscle (Klöckner and Isenberg 1994) and in rat hippocampal neurons (Church et al. 1998). In the present study, indirect effects of pH₀ changes on calcium currents via intracellular H⁺ were minimized by modest changes in pHᵢ within a physiological range and using strongly buffered pi-
pette solutions (50 mM HEPES) (Irisawa and Sato 1986; Tombaugh and Somjen 1996; Tytgat et al. 1990; Zhou and Jones 1996). Moreover, the effects of changes in pH, were always fast, which would require either appreciable proton currents or proton exchange systems for rapidly changing pH. In contrast, in an in situ slice preparation of VB we have previously shown that changes in pH due to changes of bath pH were typically slow and too small to significantly affect other pH-dependent conductances, as for instance, the hyperpolarization-activated cation current ($I_h$), present in VB neurons (Munsch and Pape 1999). In summary, these findings argue in favor of an extracellular proton modulatory effect on calcium currents when changing the pH of the bathing solution, at least in a physiological range. An extracellular proton sensitivity for calcium currents has been described in a variety of cell types (Church 1999; Irisawa and Sato 1986; Tombaugh and Somjen 1996; Tytgat et al. 1990; Zhou and Jones 1996).

### Possible mechanisms of pH sensitivity of LVA and HVA

The effects of physiologically relevant changes of pH on LVA and HVA calcium currents, described in the present paper, could in principle be due to pH-induced gating shifts or changes in channel conductance, or both. The underlying mechanisms are thought to either involve screening of fixed negative charges facing the surface of the cell membrane, which will cause a shift in the membrane potential perceived by the voltage sensor of a particular ion channel, or direct binding of H$^+$ ions to negative groups within the channel, which will interfere with current flow through the channel pore. The effects of changes in pH on activation and inactivation kinetics of both LVA and HVA calcium currents of thalamic relay neurons support the interpretation that the primary effect is due to surface charge screening by extracellular H$^+$. For heterologously expressed $\alpha 1H$ and $\alpha 1G$ currents, it was recently shown that a proton-induced change in channel
gating accounted for most of the effects of extracellular pH shifts on current amplitude (Delisle and Satin 2000; Kozlov et al. 2000). Also, the voltage dependence of deactivation of α1H currents was not affected by changes in pHo (Delisle and Satin 2000), which is consistent with a reduction of negative surface potential by protons (Hille et al. 1975). Evidence for both surface charge screening and channel block by H⁺ was found for T-type cardiac calcium channels (Tytgat et al. 1990), N-type calcium channel currents of bullfrog sympathetic neurons (Zhou and Jones 1996), and L-type Ca²⁺ channels expressed in Xenopus oocytes (Chen et al. 1996).

Differential pHo sensitivity of LVA and HVA currents

In isolated rat hippocampal CA1 neurons, a differential sensitivity to changes in pH has been observed between LVA and HVA calcium currents. LVA calcium currents were more sensitive to changes in pHo and appeared relatively insensitive to pHo changes, as revealed by exposing CA1 neurons to weak acids and bases, thereby affecting pHo, and by exposure to bathing solutions of different pHo (Tombaugh and Somjen 1997). The results of our study suggest a similar preferential pHo sensitivity of LVA calcium currents in thalamic relay neurons. However, HVA calcium currents in thalamic relay neurons were much less affected by changes in pHo than HVA Ca²⁺ channels in cortical neurons (Ou-Yang et al. 1994), hippocampal neurons (Church et al. 1998; Tombaugh and Somjen 1996), and retinal photoreceptors (Barnes et al. 1993), which is supported by a lower pKₐ value as compared with hippocampal neurons (pKₐ = 6.5, this study vs. pKₐ = 7.1, Tombaugh and Somjen 1996; and pKₐ = 7.2, Church et al. 1998). For LVA calcium currents of relay neurons, we found a pKₐ of 6.9, which is very similar to that found for the cloned T-type calcium channel subunit α1G expressed in HEK-293 cells (6.5–7.0) (Kozlov et al. 2000). Through this differential pHo sensitivity of LVA and HVA calcium currents, certain aspects of the electrogenic activity of thalamic relay neurons might be selectively modulated during activity-related pHo transients, which have been shown to accompany normal as well as pathological neuronal activity (for review see Kaila and Ransom 1998).

Functional significance for oscillatory activity in the thalamocortical network

HVA calcium currents in thalamic neurons are thought to regulate tonic firing of fast action potentials, thereby providing Ca²⁺ influx necessary for calcium-induced release of Ca²⁺ from intracellular stores (CICR) and subsequent activation of Ca²⁺-dependent K⁺ currents (Budde et al. 2000; Guyon and Lerescue 1995; Hernandez-Cruz and Pape 1989; Kamermeyer and Jones 1997). The tonic firing mode has been associated with functional states of arousal (McCormick and Bal 1997; Steriade et al. 1993). The LVA calcium current, by comparison, is thought to regulate rhythmic oscillatory patterns of electrical activity, which appears as burst discharges during slow-wave sleep or generalized epilepsy (McCormick and Bal 1997; Sherman and Guillery 1996). Recently we have shown that the two major modes of relay cell activity are accompanied by distinct patterns of intracellular pH changes that potentially contribute to the control of mode switching of relay neurons (Meyer et al. 2000). The differences in the H⁺ sensitivity of calcium channels of thalamic relay cells provide a mechanism by which certain aspects of the electrogenic behavior of relay neurons can be selectively modulated. For instance, depression of HVA calcium currents by intracellular acidosis may limit Ca²⁺ influx during tonic activity representing a negative feedback to prevent excessive Ca²⁺ influx. Such a pHo sensitivity of Ca²⁺ dynamics has recently been shown in rat CA1 neurons (Tombaugh 1998). The situation seems more complicated, however, in view of recent reports on an inhibition of Ca²⁺-dependent K⁺ current activation by a fall in pH (Church et al. 1998; Tombaugh 1998), possibly resulting in broadening of action potentials and increase in Ca²⁺ influx. The net effect of those interactions for neuronal integrative behavior in thalamus remains to be elucidated.

By comparison, the preferential pHo sensitivity of LVA calcium currents might provide a mechanism more relevant in the control of Ca²⁺ influx during burst mode. During this activity mode, relay neurons rhythmically generate LTSs (Llinás and Jahnsen 1982; Steriade et al. 1993). Since modulation of LVA calcium currents is considered an important element for rhythmic membrane potential oscillations in thalamocortical neurons (McCormick and Bal 1997), it is obvious that activity-related pHo changes potentially influence bursting behavior by affecting the generation of LTSs. Indeed, we found that extracellular acidification by 0.4 pH units drastically reduced the frequency of LTS generation in relay neurons, maintained in an in vitro slice preparation, whereas extracellular alkalization caused the opposite effect (Fig. 5). By afferent stimulation (5 s at 10 Hz) of the dorsal lateral geniculate nucleus, transient alkaline pHo shifts of 0.04 ± 0.02 pH units followed by acid shifts of 0.05 ± 0.03 pH units could be elicited in rat thalamic slices (Tong and Chesler 1999). The magnitude of these activity-dependent pHo shifts is smaller than the artificial changes in pHo used in the present study. However, the tips of ion-selective microelectrodes cause artificial enlargement of the extracellular space volume. Therefore pHo shifts recorded by ion-selective microelectrodes in brain slice preparations are likely attenuated representations of the actual pHo shifts occurring in the small extracellular space. Also, during sustained electrical activity and synchronized oscillatory activity, as occurs in the thalamocortical network under normal and pathological conditions, activity-related pHo shifts may amount to several tenths of a pH unit.

Recently, in rat CA1 hippocampal pyramidal neurons, the Ca²⁺-mediated rebound response following a current-evoked hyperpolarization was shown to be modulated by changes in pHo (Church 1999). Therefore it seems feasible to speculate that activity-related pHo transients may contribute to the control of rhythmic oscillatory activity also in the thalamocortical network. Synchronized oscillations in the thalamocortical network have been shown to be under control of a hyperpolarization-activated cation conductance as a key mechanism for the modulation of duration and frequency of network oscillations (Bal and McCormick 1996). Previously we have shown that activity-related intracellular pH shifts may contribute to the temporal control of network oscillations by modulation of the hyperpolarization-activated cation conductance (Munsch and Pape 1999). The pHo sensitivity of LVA calcium channels makes them likely candidates for an additional mechanism by which the propensity of the thalamocortical network to gener-
ate synchronized oscillations can be controlled. However, it remains to be demonstrated whether activity-related pH shifts during synchronized network activity are large enough to affect LTS generation in thalamocortical neurons. The steep pH dependence of LVA current amplitude and activation kinetics in particular at physiologically relevant pH clearly argues in favor of such a mechanism.

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