Relation Between Bicarbonate Concentration and Voltage Dependence of Sodium Currents in Freshly Isolated CA1 Neurons of the Rat

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Bruehl, C. and O. W. Witte. Relation between bicarbonate concentration and voltage dependence of sodium currents in freshly isolated CA1 neurons of the rat. J Neurophysiol 89: 2489–2498, 2003. First published December 27, 2002; 10.1152/jn.01083.2002. It recently has been shown that whole cell calcium and sodium currents are modulated by CO₂/HCO₃⁻-buffered saline. While the bicarbonate ion, but not CO₂, has been proven to modulate calcium currents, this information is lacking for sodium currents. Furthermore, it is not known whether the strength of modulation depends on the bicarbonate concentration or whether it is an all-or-nothing phenomenon. To answer these questions, we used the whole cell voltage-clamp technique on freshly isolated hippocampal CA1 neurons from the rat. A voltage step from −130 to −20 mV elicited a sodium current with an amplitude of −5.1 ± 0.5 nA (mean ± SE, n = 17) when cells were superfused with HEPES-buffered saline. The amplitude of this current increased during a subsequent superfusion with solutions containing increasing amounts of bicarbonate and CO₂ (%CO₂/ mM HCO₃⁻: 2.5/5.6; 5.0/18; 10/37), with a maximal increment in 10% CO₂/37 mM HCO₃⁻ of −6.9 ± 0.8 nA. The increase in amplitude was associated with a linear negative shift (slope: −0.7 mV/mM HCO₃⁻) of the potential of half-maximal activation (ΔV½: −19.4 ± 1.8 mV in 10% CO₂) but not with an alteration in the maximal conductance (gmax; HEPES: 203.1 ± 21.0 nS and 10% CO₂/37 mM HCO₃⁻: 207.3 ± 21.3 nS). In addition, the potential of half-maximal inactivation (V½i) shifted to more negative potentials (slope: −0.6 mV/mM HCO₃⁻) with increasing amounts of bicarbonate and CO₂ (HEPES: −53.6 ± 11.8 mV; 10% CO₂/37 mM HCO₃⁻: −69.8 ± 2.1 mV), making the amplitude of the current highly sensitive for small potential changes at resting membrane potential. The same negative shift in voltage dependence arose when cells were exposed to solutions with different amounts of bicarbonate (5.6; 18; 26 mM) but constant CO₂ (5%) with slope rates of −0.5 mV/mM HCO₃⁻ for V½a, and −0.5 mV/mM HCO₃⁻ for V½i. Again, there was no correlation between bicarbonate concentration and the size of gmax. When currents were evoked in solutions containing a constant concentration (18 mM) of bicarbonate but different amounts of CO₂ (2.5; 5.0; 10%), no significant changes have been observed. The present data demonstrate that bicarbonate ions, and not CO₂, modulate voltage-gated sodium currents in a concentration-dependent manner. Because the amplitude of the sodium current becomes highly sensitive to membrane potential changes concomitant with increased bicarbonate amounts, this may be critical for the excitability of the neuronal network in situations (like metabolic acidosis, respiratory alkalosis and hypercapnia) in which the concentration of this ion can alter.

INTRODUCTION

Solutions containing CO₂/HCO₃⁻, which act as the pH buffering system, have recently been shown to modulate whole cell calcium as well as sodium currents (Bruehl et al. 2000; Gu et al. 2000). Furthermore, the excitability of neurons in the slice preparation is different when the tissue is superfused with CO₂/HCO₃⁻-buffered saline instead of a solution buffered with the artificial pH buffer HEPES (Church 1992, 1999; Church and McLennan 1989; Cowan and Martin 1995, 1996; Gu et al. 2000). Moreover, we have demonstrated that the voltage-dependent properties of calcium currents and their maximal conductance are concentration dependent modulated when the amount of bicarbonate ions was raised from 0 up to 37 mM (Bruehl et al. 2000). Gu et al. (2000) showed a strong negative shift in the voltage dependence of voltage-gated sodium currents after the exchange of a HEPES-buffered saline for a solution buffered with 26 mM HCO₃⁻ and 5% CO₂. Moreover, they demonstrated a largely reduced excitability of the CA1 neurons during the CO₂/HCO₃⁻ situation when cells were held in current-clamp mode. Switching from a nominally CO₂/HCO₃⁻-free HEPES to a CO₂/HCO₃⁻-containing medium cannot distinguish between bicarbonate or CO₂ as the modulating factor to evoke these effects. Furthermore, it is not clear whether these alterations are an all-or-nothing phenomenon or whether they depend on the concentration/gas-pressure of the modulator (i.e., HCO₃⁻ or CO₂).

Because CO₂/HCO₃⁻ is the major pH buffer system in the CNS and alterations in the concentration of both CO₂ and HCO₃⁻ can occur during normal, as well as pathophysiological conditions, it is important to investigate whether and to what extent CO₂/HCO₃⁻-buffered saline can modulate ion conductances like the whole cell sodium current. Therefore we undertook the present study to unravel which of both components induces the modulation of the sodium current properties and whether there is a concentration dependence as can be seen on whole cell calcium currents.

For this purpose, we used the conventional whole cell voltage-clamp technique on freshly isolated hippocampal CA1 neurons of young Wistar rats.

METHODS

Cell preparation

CA1 pyramidal neurons were enzymatically isolated from male Wistar rats (50–75 g) as described in detail previously (Vreugdenhil and Wadman 1992). Slices (500 μm) were cut from both hippocampi,
and the CA1 area was dissected. These tissue pieces were incubated for 38 min at 32°C in oxygen-saturated dissociation solution (in mM/l: 120 NaCl, 5 KCl, 1 CaCl₂, 2 MgCl₂, 20 PIPES, 25 d-glucose; pH: 7.0; osmolarity: 295 mosmol/l) containing 1 mg/ml trypsin (Bovine Type XI). Subsequently, enzymatic treatment, tissue was washed twice and kept in the dissociation solution without trypsin at 19°C. Directly before measurements tissue pieces were dispersed in HEPES-buffered bath solution by triturating through Pasteur pipettes with decreasing tip diameter, and cells were allowed to settle in the perfusion chamber.

To assure total solution exchange, we used a bath chamber with a volume of ~120 μl, which was perfused with a constant flow rate of 1 ml/min. Bath solutions contained (in mM/l): 37 NaCl, 5 KCl, 2.5 CaCl₂, 1 MgCl₂, 5 4-aminopyridine (4-AP), 30 TEA-Cl, 72 choline-Cl, and 25 d-glucose and 100 μM CdCl₂; pH was set at 7.3 (unless otherwise stated), with an osmolarity of 318 mosmol/l. For seal formation, the majority of cells were patched in the preceding mentioned solution, plus 10 mM HEPES as the pH-buffer system. Bath solutions containing CO₂/HCO₃⁻ instead of HEPES were thoroughly gassed with different amounts of CO₂ (2.5; 5.0;10%) before the corresponding amount of HCO₃⁻ was added. NaCl was replaced equimolarly by NaHCO₃, and osmolarity was adjusted with glucose to 318 mosmol/l when necessary. Special care was taken to assure that the solutions were always equilibrated with CO₂ throughout the course of the experiment because otherwise CaCO₃ and CdCO₃ would have precipitated. Liquid-junction potentials may occur at the tip of the patch electrodes due to the different ion compositions of the solutions and may in some cases mislead the measurements. Therefore we measured the electrode potentials in HEPES- and bicarbonate-buffered solutions. The observed junction potentials never exceeded ±0.5 mV and were therefore regarded as negligible.

All chemicals were obtained from Sigma (Deisenhofen, Germany) or Merck (Darmstadt, Germany).

Current recording

Currents were measured under whole cell voltage-clamp conditions at room temperature using patch pipettes of 2–4 MΩ resistance. Electrode solution contained (in mM/l): 5 NaCl, 115 CsF, 2 MgCl₂, 0.5 CaCl₂, 115 TEA-Cl, 10 EGTA, 5 phosphocreatine, 2 MgATP, 0.1 NaGTP, and 0.1 leupeptin (pH set at 7.3) and 50 units/ml phosphocreatine kinase. Osmolarity was adjusted to 300 mosmol/l, when necessary, by adding glucose. The solution was heavily buffered by 50 mM HEPES to minimize intracellular pH changes following the activation of the sodium current. A combination of a third-order Boltzmann activation function and the Goldman-Hodgkin-Katz (GHK) current-voltage relation (Hille 1992; Kortekaas and Wadman 1997)

\[ I(V) = I_{\text{max}} \frac{g_{\text{max}} [\text{Na}^+]_o [\text{Na}^+]_i}{1 + \exp \left( \frac{V - V_c}{V_t} \right)} - \exp \left( \frac{-\alpha V}{\tau_\text{ext}} \right) \]

with \( \alpha = F/R/T \) and \( g_{\text{max}} = \alpha F P_0 [\text{Na}^+]_o \) where \( g_{\text{max}} \) is the maximal membrane conductance (which is proportional to the maximal permeability and the extracellular sodium concentration), \( V_c \) is the potential of half-maximal activation, and \( V_t \) is proportional to the slope of the curve at \( V_c \). F represents the Faraday constant, R the gas constant, \( P_0 \) is the maximal permeability, and \( T \) the absolute temperature.

The voltage dependence of steady-state inactivation of the sodium current was estimated from the relation of peak current amplitude versus the prepotential. This relation was well described by a Boltzmann function, which also normalized the current

\[ N(V) = \frac{I(V)}{I_{\text{max}}} \]

where \( N(V) \) is the level of steady-state inactivation determined from the current amplitude \( I(V) \) normalized to \( I_{\text{max}} \), \( V \) is the prepulse potential, \( V_c \) is the potential of half-maximal inactivation, and \( V_t \) is a factor proportional to the slope of the curve at \( V_c \).

Kinetics of the whole cell sodium currents were determined using a fit procedure which implies a third-order exponential term for activation and two exponentials describing the inactivation kinetics. The following function was applied

\[ I(t) = I_{\text{pre}} \left( 1 - \exp \left( \frac{t - \theta}{\tau_1} \right) \right) \times \left( I_1 \exp \left( \frac{t - \theta}{\tau_1} \right) + I_2 \exp \left( \frac{t - \theta}{\tau_2} \right) \right) \]

where \( I_1 \) and \( I_2 \) are the amplitudes of the two current components and \( \tau_1 \) and \( \tau_2 \) represent the time constants for activation and inactivation after the start of the voltage step at time \( \theta \).

Statistics

Values are presented as the mean ± SE. Statistical comparisons were made with Student’s t-test, if not stated otherwise. \( P < 0.05 \) was used to indicate significant differences.

RESULTS

Concentration dependent effect of CO₂/HCO₃⁻-buffered solution on whole cell sodium currents

ACTIVATION IN HEPES-BUFFERED SALINE. In a HEPES-buffered saline, whole cell sodium currents could be evoked in all
neurons (n = 16) tested (Fig. 1). They showed a fast activation and an almost complete inactivation when elicited from a potential of −130 mV (Fig. 2A, left). A voltage protocol that steps to different voltage levels (between −70 and +15 mV) revealed the typical current voltage relation (Fig. 2B, bottom) with a mean peak amplitude of −5.4 ± 0.5 nA at around −15 mV. When those I-V curves were fitted with Eq. 1, they delivered three variables, i.e., the maximal sodium conductance (g_{max}), the potential of half-maximal activation (V_{h,a}), and the slope factor at the point of V_{h,a}(V_c). For the cells tested in this way, g_{max} was evaluated to be 203.1 ± 21.0 nS (Fig. 3, left) and 50% of the channels were activated at a voltage of V_{h,a} = −27.4 ± 1.4 mV with a slope of V_c = 4.3 ± 0.3 mV (Fig. 2B, top). With regards to the different ion compositions and evaluation methods (third- vs. first-order Boltzmann activation functions), these values resembled data previously been published from freshly isolated CA1 neurons (Gu et al. 2000; Ketelaars et al. 2001).

**INACTIVATION IN HEPES-BUFFERED SALINE.** To investigate the steady-state inactivation properties, we evoked currents with a voltage step to −20 mV (10 ms) following a 500-ms period at different prepotentials (−110 to −45 mV; increment: 5 mV). When the measured peak amplitudes were plotted against the prepotential voltage, it gives a Boltzmann-like inactivation curve that was best described by Eq. 2. The values received by a fit with this equation; i.e., the half-maximal potential of steady-state inactivation (V_{h,i}) and the slope of the Boltzmann curve at this potential (V_c) amounted to −53.6 ± 1.8 and −6.1 ± 0.2 mV, respectively.

**KINETICS IN HEPES-BUFFERED SALINE.** When evoked by potential changes from −130 mV to more positive values, currents activated rapidly and inactivated almost completely within the time period of 20 ms. The kinetic of activation and inactivation could be best described using a combination of a third-order exponential term for the activation of the current and two exponential terms for the inactivating part (Eq. 3). Application of this algorithm usually leads to fit results within the noise level (Fig. 4, top). The evaluation of the time constants was restricted to test voltages of −35 up to −5 mV and delivered time constants for activation, which decreased with more positive voltages, starting with 0.39 ± 0.11 ms at −35 mV and ending with 0.08 ± 0.01 ms (n = 16) at −5 mV. The time constants for inactivation were also voltage dependent and were estimated to be in the range of 7.98 ± 1.46 ms (at −35 mV) and 3.11 ± 0.24 ms (at −5 mV) for the slow component and 1.33 ± 0.43 and 0.51 ± 0.03 ms (n = 16), for the fast inactivating component (Fig. 4, ○).

**ACTIVATION IN CO_2/HCO_3^- BUFFERED SALINE.** After performing the described protocols the bath perfusion was switched from HEPES buffer to solutions containing different amounts of CO_2 and HCO_3. Cells were subjected to solutions containing 2.5% CO_2/5.6 mM HCO_3, 5% CO_2/18 mM HCO_3, and 10% CO_2/37 mM HCO_3, with the same pH of 7.3 for all solutions. Half the cells were tested with solutions in ascending order of the amounts of CO_2 and HCO_3, while the other half were tested with solutions in descending order to avoid any hysteresis effect induced by the order of solution changes. Because the effects were fully reversible, no difference was found between the two sequences of solution exchanges. Data were pooled and are presented as mean values. No sign of a solution dependent alteration in g_{max} was observed (Fig. 3) during these experiment. None of the g_{max} values obtained in the three CO_2/HCO_3^- buffered solutions was significantly different from the value seen in HEPES-buffered solution, which could be shown by one-way ANOVA analysis (Table 1). Changing to a solution with low CO_2/HCO_3^- buffering (i.e.: 2.5% CO_2 and 5.6 mM HCO_3) following the HEPES condition induced only small alterations of the currents (Fig. 2A, top). The mean peak amplitude (at −15 mV) was merely unaffected (Fig. 2B, ▲) with a value of −5.4 ± 0.6 nA, while the potential of halffmaximal activation V_{h,a} was negatively shifted by −5.8 ± 2.1 mV (Fig. 5B1) to a voltage of −33.2 ± 2.7 mV, and the slope (V_c) was almost unchanged 3.8 ± 0.4 mV. Increasing the amounts of CO_2 and HCO_3 in the bath solution led to a more pronounced negative shift of V_{h,a} and to a significant increase of the current amplitude. The addition of the 5% CO_2/18 mM HCO_3^- buffered saline resulted in a shift of V_{h,a} by −13.1 ± 1.8 mV to −40.5 ± 2.2 mV, which was significantly different from the V_{h,a} measured in HEPES solution (one-way ANOVA: P < 0.05, Fig. 5B1), and in a raise of the peak amplitude to −6.7 ± 0.8 nA (at −25 mV). Again the slope V_c was only slightly affected (3.4 ± 0.4 mV). The shift of voltage dependence and increase in amplitude was most pronounced in the 10% CO_2/37 mM HCO_3^- containing solution, with ΔV_{h,a} : −19.4 ± 1.8 mV (V_{h,a} : −46.9 ± 1.8 mV; P < 0.05) and a peak amplitude value of −7.9 ± 1.0 nA (at −35 mV; P < 0.05). No alteration of the slope V_c (3.5 ± 0.6 mV) was observed (Figs. 2B and 5B1). The shift of V_{h,a} induced by increasing amounts of CO_2 and HCO_3, was almost linear within the concentration-range measured with a slope-rate of −0.7 mV/mM HCO_3. Nevertheless it cannot be excluded that with even higher amounts of CO_2 and HCO_3, this function will break away from linearity. A subtraction of the I-V curves obtained in HEPES-buffered solution from those in CO_2/HCO_3^- containing solutions revealed an increase in current amplitude that was
most prominent in the voltage range of −50 to −30 mV (Fig. 5A).

Despite the pronounced increase in current amplitude, no sign of a solution-dependent alteration in \( g_{\text{max}} \) was observed because the values for the three \( \text{CO}_2/\text{HCO}_3^- \)-buffered solutions were (in ascending order): 197.3 ± 20.9, 205.5 ± 22.8, and 207.3 ± 21.3 nS (Fig. 3).

**INACTIVATION IN CO\(_2\)/HCO\(_3^-\)-BUFFERED SALINE.** Concomitantly with the shift of voltage dependence of activation, also the voltage parameters of the steady-state inactivation were negatively shifted when the concentrations of \( \text{CO}_2/\text{HCO}_3^- \) were elevated (Fig. 5B). The potential of half-maximal inactivation was shifted almost linearly (within the concentration-range measured; slope-rate: −0.6 mV/mM \( \text{HCO}_3^- \)) by −2.8 ± 1.2, −8.8 ± 1.1, and −16.2 ± 1.0 mV when \( \text{CO}_2/\text{HCO}_3^- \) was added in ascending order (absolute values: −56.4 ± 1.9 mV, −62.4 ± 2.0 mV, \( P < 0.05 \); −69.8 ± 2.1 mV; \( P < 0.05 \)). As with activation, no alteration of the slope (\( V_c \)) at the point \( V_{\text{h,i}} \) was observed (Table 1). The calculation of the pairwise difference between the potentials of half-maximal activation and inactivation showed no significant difference among the solutions (−25.4 ± 2.2, −25.0 ± 2.8, −24.0 ± 2.1, and −24.0 ± 2.1 mV; \( n = 14 \)). This demonstrated that the effect took place to the same degree on the activation as well as on the inactivation properties of the currents.

**KINETICS IN CO\(_2\)/HCO\(_3^-\)-BUFFERED SALINE.** The prominent shift in voltage dependence of the current characteristics with increasing amounts of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) were accompanied by alterations in the inactivation and activation kinetics (Fig. 4, **bottom**). Both time constants for inactivation decreased when \( \text{CO}_2 \) and \( \text{HCO}_3^- \) were increased, yielding values, in the 10% \( \text{CO}_2/37 \text{mM HCO}_3^- \)-containing solution, of 4.13 ± 0.36 and 1.64 ± 0.25 ms (−35 and −5 mV; \( n = 17 \)) for the slow component and 0.64 ± 0.06 and 0.31 ± 0.03 ms for the fast current component. The time constant for activation showed qualitatively the same reduction when cells were exposed to increasing amounts of bicarbonate and \( \text{CO}_2 \) with minimal values of 0.13 ± 0.01 ms (−35 mV) and 0.12 ± 0.01 ms (−5 mV) in the solution containing 10% \( \text{CO}_2/37 \text{mM HCO}_3^- \).

**FIG. 2.** A: whole cell sodium current evoked by a voltage step from −130 to −20 mV (see inset) of a CA1 neuron. Cell was subsequently bathed in 4 solutions containing either HEPES as the pH buffer, or \( \text{CO}_2/\text{HCO}_3^- \) with different concentrations. The amplitude of this current increased in parallel with increasing amounts of bicarbonate. B: I-V relationship (**bottom**) and activation properties (**top**) of the voltage-gated sodium current in relation to the concentration of bicarbonate and \( \text{CO}_2 \). Currents are activated from a prepotential of −130 mV. The peak amplitude increased and shifted to more negative potentials, when the amount of \( \text{CO}_2/\text{HCO}_3^- \) was elevated. This phenomenon underlies a negative shift of the Boltzmann curves of activation, which results in an increased driving force for the sodium ions.
Bicarbonate but not CO₂ shifts the voltage dependence of sodium currents

In the first set of experiments, both compounds of the CO₂/HCO₃⁻/H₁₁₀₀₂ buffer were altered to keep the extracellular pH constant. It was therefore not possible to state whether CO₂ or bicarbonate modulates the whole cell sodium current. In a second series of experiments, the concentration of CO₂ was held constant at 5%, and the concentration of bicarbonate was altered (5.6; 18; 26 mM). Under these circumstances, extracellular pH varies considerably between the CO₂/HCO₃⁻-buffered solutions (pH: 6.96; 7.30; 7.44; respectively). Therefore experiments were carried out in which only one switch, from HEPES-buffered saline to the concerned CO₂/HCO₃⁻-buffered saline was performed. The pH value of the HEPES- buffered saline was adjusted to match the CO₂/HCO₃⁻-buffered saline to avoid any effect by a difference in pH₀. The concentration of the high bicarbonate containing solution (i.e., 37 mM HCO₃⁻) was reduced to 26 mM HCO₃⁻ to prevent precipitation of CaCO₃ and CdCO₃. In total, 58 neurons were investigated.

When I-V curves of the whole cell sodium current were constructed from the data obtained in HEPES-buffered solutions, a depression of the current amplitude over the entire voltage range was observed with more acidic pH₀ values (Fig. 6B). This reduction was mainly due to a slight, but not significant, decrease of $g_{\text{max}}$, concomitantly with the decreased pH₀. As previously observed (Tombaugh and Somjen 1996), only small and not significant shifts in the potentials of half-maximal activation and inactivation could be elicited. The following switch to the corresponding CO₂/HCO₃⁻-buffered solutions led to a clear evidence for a bicarbonate-concentration-dependent effect on the voltage properties of activation and inactivation.

![Figure 3](image_url)

**FIG. 3.** Bar-graph showing the maximal conductances ($g_{\text{max}}$), as were evaluated with Eq. 1 for 3 different bicarbonate and CO₂ combinations (see subtitles) and the HEPES-buffered solution. The observed increase in sodium current amplitude was not accompanied by an increase in maximal conductance when neurons ($n = 16$) were successively bathed in solutions with increasing amounts of CO₂/HCO₃⁻.

**FIG. 4.** Activation and inactivation kinetics of the whole cell sodium current. The original current (●, top) was best fitted (scattered line) with a 3rd-order exponential for activation (time constant $\tau_a$) and a double exponential for inactivation (time constants $\tau_{i,1}$ and $\tau_{i,2}$). Increasing the amounts of CO₂ and HCO₃⁻ led to a decline of the time constant of activation in the voltage range of −35 to −20 mV (left, bottom), while the time constants of inactivation decreased over the entire voltage range with increasing CO₂/ HCO₃⁻ (middle and right).
Lack of modulation by CO₂

As a complementary series of experiments, also CO₂/HCO₃⁻-buffered solutions were tested, which contained equal quantities of bicarbonate (18 mM) but different amounts of CO₂ (2.5, 5.0, and 10%). This was done to justify whether bicarbonate alone can modulate the whole cell sodium currents or whether CO₂ has an additional effect. Again the pH values of the HEPIES-buffered solutions were adjusted to fit with the values of the corresponding CO₂/HCO₃⁻-buffered saline (pH: 7.5, 7.30, 7.0). Briefly, the use of solutions with different CO₂ concentrations, but constant amounts of bicarbonate, did not change any of the current parameters in a concentration- and CO₂-dependent manner. The potential of half-maximal activation \( V_{\text{h,a}} \) was negatively shifted by about the same amount in all three solutions (Fig. 5B3) with \(-11.4 \pm 2.5\) mV (6), \(-12.8 \pm 1.6\) mV (18), and \(-8.5 \pm 1.3\) mV (6). A similar result was obtained for the half-maximal potential of inactivation (\(\Delta V_{\text{h,i}}\): 9.9 ± 1.3, 9.0 ± 1.1, and 8.2 ± 0.4 mV). Also the slope factors (\(V_\text{c} \)) of both activation and inactivation and, furthermore, the maximal sodium conductance did not differ significantly between the solutions tested. Finally, the difference (Fig. 5A3) between \(I-V\) curves obtained in HEPIES- and CO₂/HCO₃⁻-buffered solutions was similar for all three CO₂ amounts, indicating that variations of CO₂ do not modulate the voltage dependence of the whole cell sodium currents.

Effects of internal pH on whole cell sodium currents

The role of pH in the modulation of sodium currents has previously been demonstrated (Tombaugh and Somjen 1996). To estimate the fraction of effect, which could be attributed to an alteration of intracellular pH, we conducted a small experimental series in which neurons were exposed to bath solutions containing 23 mM Na-acetate. Because the acetic acid crosses the membrane like the carbonic acid, this solution mimics the acid load of the neurons, which can be assumed during exposure to CO₂/HCO₃⁻-containing solutions. The intracellular pH during these experiments was controlled by only 10 mM HEPES, instead of 50 mM, to show the maximum effect of the intracellular pH changes.

With this weak intracellular pH buffering, several changes of the sodium current properties were observed during the measurements. The maximal conductance was decreased by 23 ± 12% (112.4 ± 6.5 vs. 87.2 ± 14.9 nS; \(P = 0.11; n = 5\)), which is opposite to the effects seen with CO₂/HCO₃⁻-buffered solution, where a small, but not significant, increase was found. Negative shifts of the potentials of half-maximal activation (\(-9.6 \pm 1.6\) mV; \(n = 5\); \(P < 0.05\)) and inactivation (\(-4.3 \pm 1.0\) mV; \(P < 0.05\)) were observed when acetate was introduced. Nevertheless this shift was grossly only half the size of the maximal shift observed with CO₂/HCO₃⁻-containing solutions. Because
the shift in the voltage sensitivity and the decrease of $g_{\text{max}}$ have opposite effects on the peak size of the $I-V$ curve, only a small, but not significant, decrease of the peak amplitude was observed (Fig. 6A). Furthermore the difference current from the $I-V$ curves derived under the acetate and nonacetate condition showed a clearly different course than was seen with CO$_2$/HCO$_3^-$-buffered solution. Because in the voltage range $-40$ to $-30$ mV, the current was negative, like in the other experiments, while in the range more positive than $-25$ mV, it gained positive values.

**DISCUSSION**

**Bicarbonate modulates sodium current activation and inactivation**

The present study indicates the potential role of bicarbonate ions as a modulator of voltage dependent properties of whole cell sodium currents in CA1 neurons of the rat. Bicarbonate, in a concentration-dependent manner, shifts the potential of half-maximal activation and inactivation to more negative voltages, leaving the slopes of the activation and inactivation curves
Therefore, the sodium current amplitude and the resulting action potential will be larger with potentials more negative than about −70 mV and much smaller with potentials positive to this value. This feature of the sodium current in CO₂/HCO₃⁻-buffered saline is different from the situation in solutions with low concentrations of bicarbonate or even in HEPES-buffered saline. Under these circumstances, the potential of half-maximal inactivation is far away (approximately −54 mV) from the resting potential value, which leaves the sodium current amplitude almost independent from voltage changes around resting potential.

Previous studies have shown that the excitability of the neuronal network is reduced when brain slices or cell cultures are superfused with CO₂/HCO₃⁻-buffered saline instead of a HEPES-buffered solution (Cowan and Martin 1995, 1996; Gu et al. 2000). This observation fits with the presented data—in the case that bicarbonate is low enough—and the previous finding that whole cell calcium currents are reduced (Bruehl et al. 2000), when CO₂/HCO₃⁻ is used as the pH buffer system. The present study also points out that the excitability might be increased under certain conditions when the bicarbonate concentration is sufficiently high (Church 1992, 1999; Church and McLennan 1989) and neurons are temporarily hyperpolarized beyond resting potential. Under these conditions, the shift in activation increases the sodium current amplitude, while the steady-state inactivation has little effect. Regarding the negative shift of the potential of half-maximal activation (Vₐ₅₀), one can predict that the action potential threshold also should shift to more negative potentials with increasing concentrations of dissolved bicarbonate. Indeed, such a shift of activation threshold was found by Church and McLennan (1989) on intracellular recorded CA1 neurons in slice preparations. They found the activation threshold lowered ≤9 mV, when the bath solution was switched from 26 to 72 mM bicarbonate, and a positive shift (≤16 mV) following a switch to bicarbonate-free (HEPES-buffered) media (Church 1992). Furthermore, both studies demonstrated that neurons that were quiet in low- or bicarbonate-free media became spontaneously firing when higher bicarbonate concentrations were used. This again can be explained by a threshold for action potential firing that is closer to the resting potential.

The present study also demonstrates that the inactivation kinetics of the sodium current is strongly affected by bicarbonate. The time constants of both components decreased over the
entire voltage range measured, while the time constant of activation decreased only in the range between −35 up to −25 mV, but remained unchanged at more positive values. Consequently, the inactivation becomes more efficient in terminating the current at potentials more positive to −25 mV, for example during the generation of action potentials. The lack of a so-called overshoot of the activation potential, which occasionally occurs in vivo (Witte et al. 1996) but also in vitro (Gu et al. 2000); see there Fig. 2A), may be a consequence of this faster inactivation in CO2/bicarbonate-buffered solution because the current shuts down before the zero voltage level has been reached.

The concentration values at which bicarbonate acts on the whole cell sodium current are clearly in the physiological range observed in brain in vivo, which has been shown to be 24–26 mM during normal activity (Betz et al. 1989). During pathophysiological processes, like metabolic acidosis, respiratory alkalosis or hypercapnia this normal level can be changed by 10–15 mM in both directions. Under these circumstances, it is most likely that the excitability of the network changes, not only by pH alterations (Church 1999; Tombaugh and Sapolsky 1990, 1996, 1997) but also by differences in bicarbonate concentration in the brain tissue.

Changes of the intracellular pH

When bicarbonate/CO2-free bath solutions are exchanged by solutions containing 10 mM Na-acetate, a solution that mimics the acid introducing properties of HCO3−/CO2-buffered media, showed only subtle alterations of the currents. The intracellular pH was only weakly buffered by 10 mM HEPES during these experiments. First, the maximal conductance decrease and the mean peak amplitude was merely unchanged. Second, the potentials of half-maximal activation or inactivation showed smaller potential shifts in negative direction as was seen in HCO3−/CO2-buffered solution. Furthermore, Tombaugh and Somjen (1997) have shown that an increase of the intracellular buffering power by enhancing the HEPES concentration blunted pH-related effects on whole cell calcium currents by ≥50%. These observations indicate that the alteration of sodium currents by bicarbonate-buffered solutions are mainly related to the modulating action of the bicarbonate ions, and are only minimally contaminated by pH-related effects. Further evidence for this conclusion arises from the experiment in which bicarbonate was kept constant (at 18 mM) and CO2 was altered from 2.5 to 10%. This should alter intracellular pH values and cause cumulative changes of the sodium current properties. However, such changes of the sodium currents were not observed.

As for the modulating action of bicarbonate ions on whole cell calcium currents, we still do not know how these ions can interact with the sodium channel pores. The mechanisms underlying the modulation of sodium currents by protons were discussed earlier in detail (Hille 1992) and might help to understand the way of modulation by bicarbonate ions. First of all, modulation of the channels needs charged ions, which can interfere with the charged domains of the channel proteins. This theoretically rules out any action of the uncharged molecule CO2. In practice, our data strongly support this hypothesis. Three theories have been proposed of how protons may alter the characteristics of the sodium channels. Two titration theories that assume that protons may titrate negative surface charges or negative acid groups within the channel have been favored. The covering of the sodium ion attracting sites should end up in a decrease of the single channel conductance, which explains the reduced sodium permeability at low extracellular pH. A reduced sodium conductance has not been observed in the present study, when bicarbonate ions act as the modulator. This finding supports the third explanation, the gating theory, because an influence of the modulator on the gating properties of the channels does not result in an alteration of the conductance, but in a shift of voltage dependence of the activation and inactivation. In fact alterations of the gating properties of sodium channels by CO2/HCO3− solutions have been demonstrated by Gu et al. 2000. Taken together, the lack of effect on the conductance and the shift in activation and inactivation, shown in the present study, plus the findings of Gu makes the gating theory, the most conceivable mechanism of how bicarbonate ions act on sodium current channels. To substantiate this assumption, further studies on the single channel level and/or binding studies are necessary.

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