Effect of Extracellular $\text{HCO}_3^-$ on $\text{Na}^+$ Channel Characteristics in Hippocampal CA1 Neurons

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Gu, Xiang Q., Hang Yao, and Gabriel G. Haddad. Effect of extracellular $\text{HCO}_3^-$ on $\text{Na}^+$ channel characteristics in hippocampal CA1 neurons. J Neurophysiol 84: 2477–2483, 2000. The effect of $\text{HCO}_3^-$/$\text{CO}_2$ on membrane properties of isolated hippocampal CA1 neurons was studied with the use of the whole cell configuration of the patch-clamp technique. Neurons were acutely dissociated from 21- to 30-day-old mice. In the current-clamp mode, $\text{HCO}_3^-$/$\text{CO}_2$ significantly hyperpolarized CA1 neurons by more than 10 mV, and decreased their input resistance. In addition, the overall excitability of these neurons was lower in the presence of $\text{HCO}_3^-$/$\text{CO}_2$ than in HEPES. Spontaneous and evoked action potential firing frequency was lower in the presence of $\text{HCO}_3^-$/$\text{CO}_2$ than in its absence. In the voltage-clamp mode, both activation and steady-state inactivation of a fast $\text{Na}^+$ current were shifted in the hyperpolarized direction in such a way that the window currents were smaller in $\text{HCO}_3^-$/$\text{CO}_2$ than in HEPES. Recovery from inactivation and deactivation from the open state of the fast $\text{Na}^+$ current was slower in $\text{HCO}_3^-$/$\text{CO}_2$ than in HEPES. We conclude that $\text{HCO}_3^-$/$\text{CO}_2$ decreases the intrinsic excitability of CA1 neurons by altering not only the passive properties of the neuronal membranes but also by changing several characteristics of the fast $\text{Na}^+$ current, including activation and inactivation kinetics as well as the recovery from inactivation and deactivation.

INTRODUCTION

Most electrophysiologists use $\text{HCO}_3^-$/$\text{CO}_2$ in the extracellular solution in slice preparations but use HEPES saline in whole cell experiments. Although changing solutions from HEPES to $\text{HCO}_3^-$/$\text{CO}_2$ or vice versa is used very frequently when the role of certain membrane proteins such as those regulating $\text{Na}^+$ or $\text{H}^+$ intracellularly is investigated, the electrophysiologic alterations that occur in neurons under these two conditions are ill defined. For example, although some data are available (Church 1992; Cowan and Martin 1995, 1996; Stea and Nurse 1991), there are still gaps in our knowledge as to how excitability is altered in neurons with $\text{HCO}_3^-$/$\text{CO}_2$ and, if so, what is the basis for the alteration. Since the presence of extracellular $\text{HCO}_3^-$/$\text{CO}_2$ can activate electrogenic transporters (Brune et al. 1994; de Hurtado et al. 1995; Deitmer and Schlue 1989) and can change $\text{pH}_i$ (Bevsee and Boron 1998; Bevers et al. 1996, 1997; Bonnet et al. 1998; Saulikoski et al. 1997; Yao et al. 1999), a number of electrophysiologic changes could be expected. We therefore undertook this study to investigate the neuronal responses to the changes imposed in the extracellular milieu and focused on the potential mechanisms that underlie excitability under various conditions. Because we have used CA1 hippocampal neurons extensively in our previous studies, we chose to investigate how these particular neurons respond to the change in the extracellular fluid.

METHODS

Solutions

For the current-clamp experiments, the external HEPES solution bathing neurons contained (in mM) 130 NaCl, 3 KCl, 1 CaCl$_2$, 1 MgCl$_2$, 10 HEPES, and 10 glucose, adjusted to pH 7.4 with NaOH. The $\text{HCO}_3^-$/$\text{CO}_2$ solution contained (in mM) 125 NaCl, 3.1 KCl, 1.25 NaH$_2$PO$_4$, 2.4 CaCl$_2$, 1.3 MgCl$_2$, 26 NaHCO$_3$, and 10 glucose, bubbled with 5% of CO$_2$ and 95% of O$_2$. The internal pipette solution for the current-clamp experiments was also similar to the internal solution for the current-clamp experiments, except for CsF or CsCl instead of KCl. The HEPES-buffered solutions for the enzymatic preparation and trituration of the CA1 cells contained (in mM) 125 NaCl, 3 KCl, 1.2 MgSO$_4$, 1.25 NaH$_2$PO$_4$, 30 HEPES, and 10 glucose. Osmolarity of all solutions was adjusted to 290 mOsm. All chemicals were purchased from Sigma.

Recording criteria

PHYSIOLOGIC CRITERIA. CA1 cells were used if they had a smooth surface, a three-dimensional contour, and pyramidal shape. CA1 cells studied were from 21- to 30-day-old mice. Cells were not considered for recording when they had flat or grainy surfaces. Similar criteria have been used by us (Cummins et al. 1994) and others (Hamill et al. 1981) on freshly triturated neurons.

ELECTROPHYSIOLOGIC CRITERIA. 1) The seal resistance was measured using Ohm’s law immediately after patching each cell to assess the quality of the seal. Neurons were considered for recording if the seal resistance was >5 GΩ. 2) Only neurons with a holding current of <0.1 nA (command potential −100 mV) on establishment of the whole cell configuration were used in the study. 3) Series resistance was <10 MΩ in neurons studied. When measured in different solutions, series resistances were 6.83 ± 0.75 (mean ± SE, n = 12) in
HEPES and 5.80 ± 0.92 (n = 7) in HCO\textsubscript{3}/CO\textsubscript{2} solution. The series resistances were compensated at 80–85% level with the Axopatch 1C amplifier (Axon Instruments). Under these conditions, the error caused by uncompensated series resistances was <0.5 mV, since the cells used had a mean I桎 of <2.5 nA. To obtain adequate voltage clamp and minimize the space-clamp problem, only small neurons with short processes were used in I桎 measurement. In addition, only cells with current-voltage (I-V) curves that were smoothly graded over the voltage range of activation (approximately −50 to −10 mV) were used, as we have done in the past (O’Reilly et al. 1997).

Electrophysiologic recording

All recordings were performed at room temperature (22–24°C). Electrodes were pulled on a Flaming/Brown micropipette puller (Model P-87, Sutter Instrument) from filamented borosilicate capillary glass (1.2 mm OD, 0.69 mm ID, World Precision Instruments). The electrodes were fire-polished, and resistances were 2–5 MΩ measured in the above solutions. Action potentials (AP) were recorded in the current-clamp mode, and AP duration was measured at half voltage between AP threshold and AP peak. Rm was measured at −70 mV as a slope of the current trace evoked by a ramp voltage from −160 to 100 mV in the voltage-clamp mode. Least-square regression analysis for 100 data points was performed to derive a relationship between voltage and current. Alternatively, in current-clamp mode, and at −75 mV holding potential, a 0.01 nA hyperpolarizing current was given and the voltage was collected to calculate the Rm. Rm derived from the two methods were similar. Vm was measured in the current-clamp mode with no holding current. All values reported are represented in means ± SE.

Preparation of CA1 cells

Mice, 21–30 days old (SJL from the Jackson Laboratory) were used, and their hippocampi were removed and sliced into 7–10 transverse sections of 400 μm thick. The slices were immediately transferred to a container with 25 ml of fresh, oxygenated, and slightly stirred HEPES buffer at room temperature. After 30 min of exposure to trypsin (0.08%) and 20 min of protease (0.05%) digestion, the slices were washed several times with HEPES buffer and left in oxygenated solution. The CA1 region was then dissected out and triturated in a small volume (0.25 ml) of HEPES buffer. These studies have been approved by the Yale Animal Care and Use Committee.

RESULTS

Morphology, passive and active properties

Acutely dissociated mouse CA1 neurons were often in an isolated form (Fig. 1A) and had varied morphology. Most cells inspected had a neuronal appearance: they had a three-dimensional form and cellular processes and were elliptical, rounded, or pyramidal in shape. For consistency, we studied only pyramidal neurons.

We first studied a number of properties at rest, in the nominal absence of HCO\textsubscript{3}/CO\textsubscript{2}, i.e., in HEPES or in the presence of HCO\textsubscript{3}/CO\textsubscript{2}-buffered solutions. Most neurons studied were rather small, and the average whole cell capacitance in the absence of HCO\textsubscript{3}/CO\textsubscript{2} was 6.0 ± 0.5 pF (mean ± SE, n = 22). We also examined in detail a number of other properties. For example, with depolarizing currents of 0.1 nA and a holding voltage of −75 mV, mean AP threshold was −54.1 ± 2.1 mV, mean AP amplitude was 83.5 ± 4.7 mV, and mean AP duration was 1.9 ± 0.2 ms (n = 21). Mean Vm in these CA1 neurons was −42 ± 2 mV (n = 19; Fig. 1B), and the mean input resistance (Rm) was 696 ± 88 MΩ (n = 17; Fig. 1C).

HCO\textsubscript{3}/CO\textsubscript{2} solutions altered a number of these properties. Whole cell capacitance was 8.0 ± 0.8 pF, and this was significantly larger than in HEPES (n = 12, P = 0.02). With depolarizing currents, mean AP threshold was −54.1 ± 2.2 mV (n = 8), with an average AP amplitude of 77.4 ± 8.7 mV (n = 8) and a mean AP duration of 1.5 ± 0.1 ms (n = 8). HCO\textsubscript{3}/CO\textsubscript{2} significantly hyperpolarized CA1 neurons (mean Vm = −53 ± 4 mV, n = 18, P = 0.004; Fig. 1B) and decreased Rm (mean Rm = 385 ± 46 MΩ, n = 21, P = 0.01; Fig. 1C). Thus HCO\textsubscript{3}/CO\textsubscript{2} solutions increased cell capacitance, rendered Vm more negative, and decreased Rm.

To test whether an electrogenic HCO\textsubscript{3}− mechanism was involved in hyperpolarizing the cells in the presence of HCO\textsubscript{3}/CO\textsubscript{2}, we used DIDS to inhibit transporters that depend on HCO\textsubscript{3}−. In the presence of HCO\textsubscript{3}/CO\textsubscript{2}, DIDS (0.5 mM) caused 5.6 ± 1.0 mV (n = 5) depolarization, and this depolarization was reversible when DIDS was removed.

Neuronal excitability in the presence and absence of HCO\textsubscript{3}/CO\textsubscript{2}

SPONTANEOUS FIRING. The effect of HCO\textsubscript{3}/CO\textsubscript{2} on spontaneous firing was studied in detail in 12 CA1 neurons. Figure 2A shows a CA1 neuron firing APs at a relatively low rate in

FIG. 1. A: morphology of freshly dissociated mouse CA1 hippocampal neuron. The cell body was usually longer than 10 μm diam. Magnification: ×600. B and C: effect of HCO\textsubscript{3}/CO\textsubscript{2} on Vm and Rm. In the presence of HCO\textsubscript{3}/CO\textsubscript{2}, both Vm and Rm decreased significantly (P = 0.004 and 0.01, respectively).
bicarbonate solution. When switched from HCO$_3^-$/CO$_2$ to HEPES solution, the same neuron fired APs at a much higher rate. When the perfusing solution was changed back to HCO$_3^-$/CO$_2$ solution, the neuron fired at a lower rate again. Indeed, although CA1 neurons had an irregular pattern of firing, they had generally spontaneous APs in HEPES. However, in all CA1 cells studied, they either did not have any spontaneous APs in HCO$_3^-$/CO$_2$ solution, or their firing rate was much lower than that in HEPES solution.

**EVOLED FIRING OF APs.** Forty-two CA1 neurons were studied, and all fired APs when they were held at $-75$ mV and given depolarizing currents in the current-clamp mode. For example, in Fig. 2B, a 0.05 nA depolarizing current evoked nine APs in this particular neuron in the absence of HCO$_3^-$/CO$_2$. When the solution was changed to HCO$_3^-$/CO$_2$, two APs were evoked in this same neuron. Switching back to HEPES reversed the inhibition, and the number of evoked APs increased to four. In some neurons, firing was totally eliminated when exposed to HCO$_3^-$/CO$_2$. APs in all neurons reappeared with the removal of HCO$_3^-$/CO$_2$. On average, the number of APs evoked by depolarizing currents of 0.1 nA was reduced from $3.9 \pm 0.9$ ($n = 21$) in the absence of HCO$_3^-$/CO$_2$ to $1.6 \pm 0.4$ ($n = 8$) in HCO$_3^-$/CO$_2$ solution. Also, less current was needed to generate the same numbers of APs in the absence than in the presence of HCO$_3^-$/CO$_2$. In Fig. 2C, the rheobase was much smaller in the absence of HCO$_3^-$/CO$_2$, whereas $147.5 \pm 35.5$ pA ($n = 12$, $P = 0.02$) were needed in HCO$_3^-$/CO$_2$. Similarly, to evoke two, three, four, or more APs, the currents used were always smaller in the absence of HCO$_3^-$/CO$_2$ than in the presence of HCO$_3^-$/CO$_2$. Clearly, one explanation for the decrease in excitability seen in neurons in HCO$_3^-$/CO$_2$ is related to the hyperpolarization and decrease in $R_m$. Note, however, that in Fig. 2B, AP generation in the presence or absence of HCO$_3^-$/CO$_2$ was induced from the same membrane potential, i.e., $-75$ mV. Therefore during evoked stimulation, the difference in excitability observed in HCO$_3^-$/CO$_2$ and in HEPES cannot be attributed to differences in $V_m$ since we controlled that variable. The drop in $R_m$ with HCO$_3^-$/CO$_2$ could, by itself, contribute to the decrease in excitability.

**Fast Na$^+$ current**

Since evoked firing of CA1 neurons was lower in the presence than in the absence of HCO$_3^-$/CO$_2$ in spite of the fact that $V_m$ was held at the same level, we raised the question as to whether the presence of HCO$_3^-$/CO$_2$ affected other membrane properties. For example, Na$^+$ channel properties and kinetics would be important to examine and could have been altered. If this were the case, we would suspect that HCO$_3^-$/CO$_2$ would change the properties of Na$^+$ channels in such a way to make CA1 neurons less excitable.

When we held CA1 neurons at $-130$ mV, depolarizing pulses to $-20$ mV evoked an inward current that reached a peak in less than a millisecond and decayed subsequently to zero current (Fig. 3A). This inward current was carried by Na$^+$ since TTX (1 $\mu$M) blocked the current almost totally (data not shown). Based on its voltage dependency, characteristics of fast activation and TTX sensitivity, we considered this as a voltage-sensitive fast Na$^+$ current. Switching solutions from the nominal absence of HCO$_3^-$/CO$_2$ to one containing HCO$_3^-$/CO$_2$ changed neither the size of the fast Na$^+$ current of CA1 neurons nor the fast Na$^+$ current density (peak current/capacitance; data not shown).

**Na$^+$ ACTIVATION CHARACTERISTICS.** With CA1 neurons held at $-130$ mV, depolarizing voltages were given from $-70$ to $80$ mV for $48$ ms at increments of $10$ mV. In the absence and presence of HCO$_3^-$/CO$_2$, the threshold for Na$^+$ current activation was about $-60$ mV (Fig. 3B, ■). The Na$^+$ currents increased in amplitude with depolarizing voltages and reached a peak at about $-20$ mV in HEPES and $-30$ mV in HCO$_3^-$/CO$_2$. The normalized conductance, plotted against $V_m$, showed a much more hyperpolarized midpoint ($m_{n,1/2}$) in the presence
STEADY-STATE INACTIVATION CHARACTERISTICS OF NA\textsuperscript{+} CURRENTS. Steady-state inactivation of Na\textsuperscript{+} currents was studied with current traces obtained using a prepulse potential from −130 to −20 mV for 502 ms and then stepping \(V_m\) to −20 mV in the absence and presence of HCO\textsubscript{3}/CO\textsubscript{2}. Figure 4, A and B, shows an example of current traces from the same cell under voltage clamp. When the normalized current \((I/I_{\text{max}})\) was plotted against the prepulse potential, the relationship could be fitted with a Boltzmann equation. The midpoints of the steady-state inactivation curves \(h_{\text{1/2}}\) were −63 and −83 mV in the absence and presence of HCO\textsubscript{3}/CO\textsubscript{2}, respectively. Note that the maximum currents were obtained from prepulse of −130 to −90 mV \((n = 6)\). The amplitude of the Na\textsuperscript{+} currents was decreased with more depolarized prepulse potentials, ranging from −90 to −40 mV. At voltages more depolarized than −40 mV, little or no Na\textsuperscript{+} current was generated in either solutions. Changing solution from HEPES to HCO\textsubscript{3}/CO\textsubscript{2}, shifted \(h_{\text{1/2}}\) in the hyperpolarized direction by about 20 mV. The \(h_{\text{1/2}}\) was −63 and −83 mV in the absence and presence of HCO\textsubscript{3}/CO\textsubscript{2}, respectively \((n = 6)\). As can be seen from the above results, since the activation curve is shifted by about 11 mV and the steady-state inactivation by about 20 mV in the hyperpolarized direction when the extracellular solution is changed to HCO\textsubscript{3}/CO\textsubscript{2}, the window currents became narrower in HCO\textsubscript{3}/CO\textsubscript{2} than in HEPES solution (Fig. 5).

![Figure 3](image-url)  
**FIG. 3.** Effect of HCO\textsubscript{3}/CO\textsubscript{2} on Na\textsuperscript{+} current activation. A: typical current traces collected from −70 to 80 mV with a 10-mV increment from a holding potential of −130 mV in the voltage-clamp mode in HEPES. B: the average of normalized currents against \(V_m\) in HEPES and in HCO\textsubscript{3}/CO\textsubscript{2}. C: activation characteristics of the Na\textsuperscript{+} current as shown by the relationship between voltage and the normalized conductance in HEPES and HCO\textsubscript{3}/CO\textsubscript{2}. Curve fittings in C were obtained from the averaged data using the Boltzmann equation.

![Figure 4](image-url)  
**FIG. 4.** Effect of HCO\textsubscript{3}/CO\textsubscript{2} on steady-state inactivation of the Na\textsuperscript{+} current. A: currents were collected at −20 mV from prepulse potentials from −130 to −20 mV with an increment of 10 mV and a duration of 502 ms. B: steady-state inactivation curves were plotted as \(I/I_{\text{max}}\) against prepulse potential and fitted by a Boltzmann equation for the averaged, normalized current against the prepulse potentials.

![Figure 5](image-url)  
**FIG. 5.** Activation and inactivation characteristics of Na\textsuperscript{+} currents in the absence and in the presence of HCO\textsubscript{3}/CO\textsubscript{2}. The curves are from Figs. 3C and 4C.
DEACTIVATION CHARACTERISTICS OF Na⁺ CURRENTS. We also examined the effect of HCO₃⁻/CO₂ on the transition from the open to the resting closed state. We held CA1 neurons at −100 mV, depolarized them for 1 ms to −10 mV, and repolarized to −70 or −100 mV. In HEPES and HCO₃⁻/CO₂, the repolarizing voltage to −100 mV evoked inward currents that decayed to zero rapidly. When we repolarized neurons to −70 mV, the decay of the evoked currents was much slower. The averaged decay constants at −100 mV were 0.18 ± 0.02 (n = 4) in HEPES solution and 0.23 ± 0.05 (n = 4) in HCO₃⁻/CO₂. At −70 mV, the averaged decay constant was much slower (0.88 ± 0.20, n = 4) in the presence of HCO₃⁻/CO₂ than in HEPES (0.29 ± 0.06, n = 4; P = 0.03). Thus HCO₃⁻/CO₂ significantly increased the time constant of deactivation of the fast Na⁺ channel in CA1 neurons when they were repolarized to −70 mV after a brief depolarization.

DISCUSSION

Although there have been a few studies detailing the effect of a change in intracellular pH on the activity of exchangers and some of the neuronal properties (Bevensee and Boron 1998; Bevensee et al. 1996, 1997; Bonnet et al. 1998; Brooks and Bachelard 1992; Church 1992; Cowan and Martin 1995; Dart and Vaughan-Jones 1992; de Hurtado et al. 1995; Deitmer 1992; Gaillard and Dupont 1990; Saarikoski et al. 1997; Yao et al. 1999), there has not been a comprehensive investigation on the role of HCO₃⁻/CO₂ bathing neurons in neuronal excitability and the basis for it. In this work we have made two observations, both of which contribute to the decrease in neuronal excitability with HCO₃⁻/CO₂. Although the extracellular pH is kept at the same level, the presence of HCO₃⁻/CO₂ in the extracellular milieu alters profoundly 1) the passive neuronal membrane properties and 2) the Na⁺ channel kinetics.

MEMBRANE PROPERTIES AND NEURONAL EXCITABILITY IN THE PRESENCE OF HCO₃⁻/CO₂. It is clear from our studies that excitability decreases in the presence of HCO₃⁻/CO₂. The rheobase in these CA1 neurons increased, and, with the same amount of current injected, the number of action potentials fired with HCO₃⁻/CO₂ was lower than that with HEPES. One major reason for this decrease in excitability with HCO₃⁻/CO₂ is the decrease in Vᵱ (more negative) and decrease in Rᵢ. With respect to Vᵱ, we believe that it is higher because of the effect of HCO₃⁻/CO₂ on possibly a number of membrane proteins, the activity of which depends on the presence of HCO₃⁻. Although intracellular pH (pHᵱ) increases with HCO₃⁻/CO₂ (Yao et al. 1999) in these CA1 neurons, clearly, the difference in proton concentrations between inside and outside of the cell cannot explain the change in Vᵱ with HCO₃⁻/CO₂ by the Nernst equation. Hence other factors are important, and we hypothesized that it is the activation of one or more electrogenic, HCO₃⁻-dependent transporters that are at the basis of the change in Vᵱ. Indeed, we have shown that DIDS, a blocker of HCO₃⁻-dependent transporters, depolarized these CA1 cells. Hence, we have evidence that HCO₃⁻ transporters play a role in the electrogenicity of these cells. Although it has been assumed that this co-transporter exists only in glia, we have recently shown that this membrane protein is also present in neurons and in the neurons we have studied in this work (Schmitt et al. 2000). While it is possible that HCO₃⁻/CO₂ activates more than one membrane protein, the net result observed can be explained only by implicating the activation of at least one such electrogenic membrane protein. It is important to keep in mind, however, that HCO₃⁻ can activate both acid extruders and

FIG. 6. Effect of HCO₃⁻/CO₂ on the recovery from inactivation of the Na⁺ current. The 2-pulse voltage protocol was shown under the 2nd trace of A. Two identical pulses were delivered with increasing intervals (t) in between the 2 pulses. B: the ratio of the current evoked from the 2nd pulse over the one induced by the 1st was plotted against the interval when neurons were exposed to HEPES and HCO₃⁻/CO₂ (n = 5). Statistical significance is shown on the plot.
loaders, with some electrogenic and others not. It is clearly possible that the activation of any number of these transporters could increase the conductance of these neurons, as we have observed in our studies. Furthermore, it is worth mentioning that the effect of HCO$_3^-$/CO$_2$ on $V_m$ depends clearly on the membrane protein endowment of the neuron; therefore it is likely that the effect of HCO$_3^-$/CO$_2$ varies from one cell type to another.

With respect to the change in $R_m$, it is possible that the presence of HCO$_3^-$/CO$_2$ induces the activation of conductances such as those of Cl$^-$ or K$^+$ that would decrease $R_m$. For example, it was reported that in glomus cells, a fivefold increase in input conductance and an opening of a putative large conductance (300 pS) anion channel (blocked by 9-AC) was induced by HCO$_3^-$/CO$_2$ (Stea and Nurse 1991), suggesting the involvement of Cl$^-$ channels. Another study showed that the depolarization induced by switching extracellular solution from HCO$_3^-$/CO$_2$ to HEPES can be blocked by 4-AP, suggesting the involvement of K$^+$ channels (Cowan and Martin 1996). Although we have shown in our studies that DIDS depolarizes CA1 cells, it is still possible that K$^+$ channels are involved in the hyperpolarization seen.

We have noticed that, in the presence of HCO$_3^-$/CO$_2$, the membrane capacitance increased significantly. At this stage, we do not have any mechanistic explanation for the increase. However, it is important to mention that similar results were observed by Rich et al. (1990, 1991), who showed that the stimulation of HCO$_3^-$-dependent mechanisms was accompanied by a simultaneous increase in apical membrane capacitance. Interestingly, the same authors also found that the apical membrane conductance was simultaneously increased.

**Na$^+$ CHANNELS AND NEURONAL EXCITABILITY IN THE PRESENCE OF HCO$_3^-$/CO$_2$.** In this study, although HCO$_3^-$ increased $V_m$, our electrophysiological pulse paradigm clamped cells at the same potential, irrespective of the bathing solution. In spite of clamping $V_m$, cells were still less excitable in HCO$_3^-$/CO$_2$. The increase in conductance could still contribute to the decreased neuronal excitability, but we found in this work that Na$^+$ channel kinetics are altered in such a way to lessen excitability.

Several alterations in Na$^+$ channel kinetics were induced with HCO$_3^-$/CO$_2$. It shifted both the conductance-voltage relation as well as the steady-state inactivation curve in the hyperpolarized direction but shifted the latter to a much larger degree (Fig. 5). The shift in the inactivation curve would indicate that, at any particular voltage on the inactivation curve, the current generated would be smaller, since there are many fewer Na$^+$ channels that can be recruited in the presence of HCO$_3^-$/CO$_2$ than in its absence. The window currents, which define the voltage range in which the Na$^+$ channels that are not fully inactivated yet can be activated, are much narrower in the presence than in the absence of HCO$_3^-$/CO$_2$. We do not believe that the change in pH$_i$ that occurs in our cells (Yao et al. 1999) (a rather small increase of 0.14 in the presence of HCO$_3^-$/CO$_2$) was the main factor causing the shift of both the conductance-voltage relation as well as the steady-state inactivation curve. As shown by other investigators (Cummins et al. 1993), increasing pH$_i$ from 6.8 to 7.3 (a much larger increase in pH$_i$ than the increase in our current study) did not have any effect on the conductance-voltage relation and shifted the steady-state inactivation curve in the hyperpolarization direction by only 5 mV.

In our case, if pH$_i$ contributed to the shift in the steady-state inactivation, it would most likely be a small one. The reasons for the alteration observed in the steady-state inactivation in the presence of HCO$_3^-$/CO$_2$ but not in its absence is not clear at present. We speculate that changes in Na$^+$ channel kinetics in response to HCO$_3^-$/CO$_2$ may include cytosolic effectors, such as kinases, which are known to have the Na$^+$ channel as their targets.

Other changes in the Na$^+$ channel kinetics can also contribute to the decrease in excitability in the presence of HCO$_3^-$/CO$_2$. For example, the time constant for the recovery from inactivation of the Na$^+$ currents was significantly larger in the presence of HCO$_3^-$/CO$_2$ than in HEPES. Therefore compared with the Na$^+$ channels in HEPES, Na$^+$ channels in HCO$_3^-$/CO$_2$ spend a much longer time in the transition from the inactivation to the closed state. Similarly, deactivation of Na$^+$ currents is significantly slower in the presence of HCO$_3^-$/CO$_2$ than in HEPES. Hence, Na$^+$ channels spend a much longer time in the open state than in the closed state when they are activated briefly and repolarized to $-70$ mV in the presence than in the absence of HCO$_3^-$/CO$_2$.

In summary, HCO$_3^-$/CO$_2$ affects a number of functional aspects in neurons that, in turn, influence excitability. Besides the change that HCO$_3^-$/CO$_2$ induces in $V_m$, $R_m$, HCO$_3^-$/CO$_2$ alters Na$^+$ channel kinetics in various ways. It would seem from our data that HCO$_3^-$/CO$_2$ decreases the availability of the Na$^+$ channel for recruitment, increases the probability of the channel to be in the inactivated state after activation, and favors the open state rather than the closed state after a brief activation.

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