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Editorial Comment

Towards bridging the gap between acid-base transporters and neuronal excitability modulation

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Abstract: pH homeostasis is a fundamental regulator of the function of the central nervous system. Dysfunction of acid-base transporters often results in disturbance of neuronal excitability. In a latest issue of *Journal of Neuroscience*, Jones *et al.* report that increasing intracellular bicarbonate concentration substantially stimulates the excitability of pyramidal neurons from mouse hippocampus by inhibiting KCNQ potassium channel. The finding shed important new light in understanding the molecular mechanism underlying the regulation of neuronal excitability by acid-base transporters.

Keywords: SLC4 family, SLC9, bicarbonate transporter, NDCBE, NHE3, acid-base balance, pH regulation, neuronal excitability, neuron, KCNQ, Kv7

Intracellular pH (pH_i) plays a fundamental role in regulating the function of the central nervous system (CNS). Except for chemosensitive neurons which are stimulated by acidosis to enhance exhalation of CO_2 [1], the excitability of most neurons in the CNS is generally upregulated by cellular alkalosis, and down-regulated by cellular acidosis (see review [2, 3]). For example, respiratory alkalosis caused by hyperthermia can significantly increase neuronal excitability, therefore increase seizure incidence [4]. In contrast, respiratory acidosis induced by CO_2 inhalation has the opposite effects on neuronal excitability [5]. On the other hand, neuronal activities, such as presynaptic transmitter release, $GABA_A$ receptor activities, action potential firing, can cause substantial transients in local pH (both pH_i and extracellular pH_o) in the CNS (for review, see ref. [6]).

The pH homeostasis is dependent on the fine balance of the activities of acid-loaders and acid-extruders. The acid loaders, such as the Na^+-independent anion exchangers of the solute carrier family 4 (SLC4), mediate the net infflux of equivalents of protons [7]. The acid extruders, such as the Na^+-H^+ exchangers of SLC9 family and the Na^+-dependent HCO_3^- transporters of SLC4 family, mediate the net efflux of equivalents of protons [8, 9]. These acid-base transporters are widely expressed throughout of the CNS and play critical roles in the regulation of pH homeostasis in the brain.

Not surprisingly, dysfunctions of these acid-base transporters are associated with a series of nervous system disorders, such as mental retardation, epilepsy, migraine, autism [10-15]. For instance, mutations in the electrogenic Na+/HCO3- cotransporter NBCe1 (SLC4A4), which is highly expressed in astrocytes and likely to a lesser extent in neurons [16], have been associated with migraine [10, 17, 18]. Targeted disruption in the electroneutral Na+/HCO3- cotransporter NBCn2 (Slc4a10), which is highly expressed in neurons throughout of the CNS [14, 19], reduces the neuronal excitability, therefore increases the seizure threshold in mice [14]. A natural mutant of the anion exchanger AE3 (SLC4A3) is associated with epilepsy in human [11]. Consistently, targeted disruption of AE3 increases the neuronal excitability and decreases the seizure threshold in mice [15]. Deficiency in NHE1 (Slc9a1) causes enhanced neuronal excitability and increased seizure incidence in mice [20, 21].

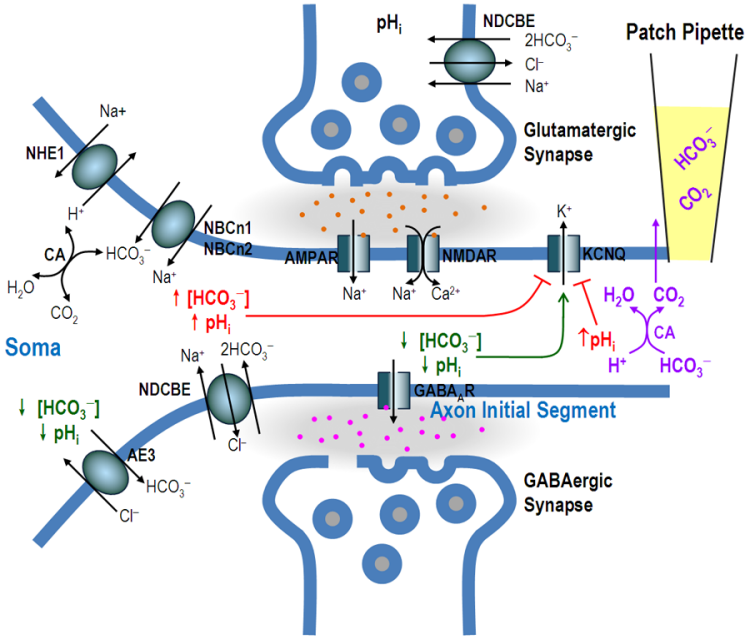

Figure 1. Acid-base transporters in neuronal excitability regulation in the CNS. At the presynaptic terminal, the activity of acid-extruder NDCBE tends to increase the intracellular [HCO_3^-] and pH_i, an effect stimulatory to the spotaneous glutamate release and therefore to the neuronal excitability. At the postsynaptic terminal, the activities of the acid-extruders (such as NHE1, NBCn1, NDCBE, and NBCn2) tend to increase the intracellular [HCO_3^-] and pH_i, an effect inhibitory to the activation of KCNQ channels. The inhibition of KCNQ would enhance the neuronal excitability. The activity of acid-loader AE3 would have the opposite effect on the neuronal excitability. The purple shows that, in the absence of extracellular CO_2/HCO_3^- in the patch clamp experiments by Jones et al [23], the intracellular CO_2 introduced by the patch pipette would flux out of the cell, a process that would cause akalosis to the pH_i.

The above demonstrations have well established the physiological and pathological significance of the acid-base transporters in the regulation of neuronal excitability. Theoretically, pH homeostasis can affect the events at both the presynaptic and the postsynaptic terminals during the neuronal signaling in the CNS. In the presynaptic membrane, it has been shown that the genetic disruption of *Slc4a8* encoding the Na^+-driven Cl^-/HCO_3^- exchanger NDCBE impairs the spotaneous glutamate release in hippopcampal neurons from mice [22]. Accordingly, the neuronal excitability in the *Slc4a8*-null mice is reduced and seizure threshold is increased.

In the postsynaptic membrane, pH homeostasis could affect the activity of neurotransmitter receptors and the gating of voltage-sensitive ion channels involved in postsynaptic response to neurotransmitter release. In a latest issue of *Journal of Neuroscience*, Jones *et al.* examined the effect of manipulating intracellular HCO_3^- content on neuronal excitability by using whole-cell patch clamp with hippocampal slices from mice [23]. The authors demonstrated that increasing the pipette HCO_3^- concentration from 0 mM to 26 mM substantially increases the action potential firing rates by hippocampal pyramidal neurons. This stimulatory effect of HCO_3^- is likely due to the inhibition of potassium channel KCNQ (*aka* Kv7), via a mechanism likely involving phosphatidylinositol-4,5-biphosphate PIP2. Jones *et al.* found that the application of KCNQ agonist retigabine totally abolishes the stimulatory effect of HCO_3^- on the action potential firing rates [23]. KCNQs is a group of M-type K^+ channels that are widely expressed in neurons in the CNS [24]. The activation of KCNQ inhibits action potential firing by opposing the membrane depolarization.

Jones *et al.* concluded that the stimulatory effect of HCO_3^- on neuronal excitability is independent of pH_i changes [23]. However, this conclusion appears to be arbitrary. The authors claim that, in their patch clamp experiments, they carefully controlled the pH of the pipette solution attempting to minimize the alteration in the neuronal pH_i [23]. However, we should note that, in the experiments to test the effect of intracellular HCO_3^-, the authors had no CO_2/HCO_3^- in the bath solution. As shown in **Figure 1**, in the absence of extracellular CO_2/HCO_3^-, the intracellular CO_2 introduced by the patch pipette would quickly flux out of the cell. The reduction in intracellular CO_2 content would then be replenished by the dehydration of HCO_3^- catalyzed by carbonic anhydrase, a process that consumes proton [25]. The intracellular HCO_3^- could also flux out of the cell via the HCO_3^- transporters. However, the rate of CO_2 efflux would be much higher than that of the HCO_3^- efflux. Therefore, under the experimental conditions, the load of CO_2/HCO_3^- by a patch pipette

would cause a great intracellular alkalosis to the neuron.

The authors also observed that, in the absence of CO_2/HCO_3^-, simply elevating the pH_i by manipulating the pH of pipette solution had no effect on the action potential firing rates by the hippocampal pyramidal neurons. However, this is a non-physiological condition with no CO_2/HCO_3^-.

The HCO_3^--induced stimulation to neuronal excitability observed by Jones *et al.* could be explained by: (1) simply an effect of HCO_3^- per se. However, one should keep in mind that, any change in intracellular [HCO_3^-] would be associated with a change in pH_i under physiological condition; (2) an effect of pH_i that requires the presence of CO_2/HCO_3^-. Given the pH-sensitivity of a number of ion channels (including KCNQ) involved in neuronal excitability, it is not likely to completely rule out a contribution of pH_i in the HCO_3^--induced stimulatory effect on neuronal excitability.

Indeed, KCNQ channels exhibit complex pH sensitivity. For example, when heterologously expressed in human embryonic kidney cells HEK293, KCNQ2/KCNQ3, the isoforms that are predominantly expressed in the CNS, are strongly activated by extracellular alkalosis [26]. This alkalosis-induced activation on the heterologously expressed KCNQ channels appears to be in the opposite direction as we would expect to be (i.e., inhibition by alkalosis) according to the findings by Jones *et al*. However, we should note that, the pH sensitivity of KCNQ channels likely depends on the compositon of the KCNQ channels. For instance, homomeric KCNQ1 is inhibited by extracellular acidosis [27], whereas KCNE1/KCNQ1 and KCNE2/KCNQ1 are activated by acidosis in a beta-subunit dependent manner [28, 29].

Nevertheless, the findings by Jones *et al.* are meaningful in the context of the modulation of neronal excitability by acid-base transporters. As shown in **Figure 1**, the acid-loader AE3 mediates HCO_3^- efflux in exchange of extracellular Cl^-. The effect of AE3 activity would be to decrease the intracellular [HCO_3^-] and lower pH_i, which in turn would enhance KCNQ activation and reduce the neuronal excitability. Disruption in AE3 would increase the intracellular [HCO_3^-] and pH_i, therefore inhibit the activation of KCNQ and enhance the neuronal excitability. Consistent with this model are the observations from human patients carrying mutation in *SLC4A3* and mice with *Slc4a3* disrupted that have increased neuronal excitability [11, 15].

Conversely, the activities of acid-extruders such as NHE1, NBCn1, NBCn2, and NDCBE would increase the intracellular [HCO_3^-] and raise pH_i, therefore attenuate the activity of KCNQ, which in turn will enhance the excitability of the neuronal network. Consistent with this model are the observations of reduced neuronal excitability from mice disrupted with NHE1 or NBCn2 [10, 20, 21].

As discussed by Jones *et al.*, their findings might also be physiologically relavent in the context of GABAergic signaling at the axon initial segment (AIS) of some axon-axonic cells. The HCO_3^- efflux through the $GABA_A$ receptor channels could substantially decrease the intracellular [HCO_3^-] and then pH_i at the AIS, which in turn would stimulate the KCNQ channel activation, resulting in an inhibitory effect on neuronal excitability. This could explain the likely inhibitory effect of $GABA_A$ receptors at the AIS of some neurons where $GABA_A$ receptor activation results in a depolarization rather than a hyperpolarization [30, 31].

In summary, the intracellular pH homeostasis in the CNS is established by the delicate balance of acid-loaders and acid-extruders. Disturbance in acid-base balance are often associated with alterations in neuronal excitability in the CNS. A number of acid-base transporters have been shown to be involved in the regulation of the neuronal excitability. The study by Jones *et al.* shed important new light on the investigation of the molecular mechanisms underlying the regulation of neuronal excitability by acid-base transporters.

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