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Review

Minireview: pH and synaptic transmission

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ABSTRACT

As a general rule a rise in pH increases neuronal activity, whereas it is dampened by a fall of pH. Neuronal activity per se also challenges pH homeostasis by the increase of metabolic acid equivalents. Moreover, the negative membrane potential of neurons promotes the intracellular accumulation of protons. Synaptic key players such as glutamate receptors or voltage-gated calcium channels show strong pH dependence and effects of pH gradients on synaptic processes are well known. However, the processes and mechanisms that allow controlling the pH in synaptic structures and how these mechanisms contribute to normal synaptic function are only beginning to be resolved.

1. Introduction

The strong acidification of synaptic vesicles by the vacuolar H^+-ATPase, which energizes the neurotransmitter loading of synaptic vesicles [1], is a main reason for the large fluctuations in synaptic pH. Synaptic vesicle exocytosis results in the release of protons into the synaptic cleft as well as in the incorporation of the vacuolar H^+-ATPase into the presynaptic membrane. Thus synaptic transmission causes a relatively short but strong acidification of the synaptic cleft [2–4]. The extracellular acidosis is subsequently followed by a long, yet transient increase in extrasynaptic pH [5]. In the hippocampus this alkaline transient can be detected within milliseconds [6,7] and reaches magnitudes as large as 0.1–0.2 pH units [8]. Mechanisms underlying this rise in pH are not fully understood but most likely presynaptic Ca^{2+}/H^+-ATPase [9,10], extracellular carbonic anhydrases [8], and $GABA_A$-receptor mediated bicarbonate efflux [11] are involved. Increased synaptic/neuronal activity can also cause a prolonged extracellular acidification because of the increased cell metabolism [5,12,13].

Although several studies have successfully monitored neuronal pH shifts in the brain [2,14,15], only very little is known about pH transients in neuronal microdomains because of technical limitations [16,17]. Direct experimental data on pH fluctuations and pH regulation in intracellular synaptic compartments so far have only been obtained for motor endplates because of their significantly larger dimensions compared to central synapses [4,18]. Zhang et al. used the pH-sensitive properties of the yellow fluorescent protein to analyse the presynaptic pH in mouse motor endplates. This study not only supports the importance of presynaptic pH regulators but further provided evidence that the release of vesicles in the peripheral nervous system is accompanied by a transient intracellular acidification. Here, the increase in pH was mainly caused by the activation of plasma membrane Ca^{2+}/H^+-ATPase and was followed by an unexpected, longer lasting alkalinisation is due to the transient incorporation of the vacuolar H^+-ATPase into the presynaptic membrane [4]. Focal injections of BCECF-AM in combination with slice imaging as used for measuring calcium transients in small synaptic compartments with the calcium-sensitive dye Fura [19], genetically encoded pH indicators [18], which also allow ratiometric imaging [20,21], may help to establish adequate and fast pH measurement in small compartments like central pre- and postsynaptic terminals in the future.

Despite these technical limitations the occurrence of rather large, spatially and timely limited, pH fluctuations in the different synaptic compartments is generally accepted and clearly implies that pH regulatory elements are essential to maintain proper synaptic function. Since many synaptic elements are strongly pH dependent, limitations and alterations in synaptic pH homeostasis could potentially feed-back on neuronal activity itself. Intriguingly,

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it has already been shown that direct release of protons during vesicle exocytosis can act as a negative feedback on closely associated calcium channels in the mammalian retina [3,22]. In this system, synaptic cleft acidification of retinal cells is thought to underlie surround inhibition and thereby helps to form the receptive field (for review see [23]). The discovery of acid-sensing ion channels (ASICs) is another example for a pH-induced feedback mechanisms [24]. At least four genes and their alternatively spliced transcripts code for subunits of such ion channels, which belong to the degenerin/epithelial Na^+ channel superfamily and are characterized by a strong H^+-sensitivity as well as their permeability for cations. ASICs are widely expressed in the mammalian nervous system and have been shown to localize mostly to somato-dendritic regions of neurons [25,26]. ASICs have been implicated in many neurological disorders like e.g., ischemic stroke, epileptic seizures and pain (for review see [27]). Interestingly, one study suggested that seizure termination critically depends on ASIC activation by the fall in extracellular pH in response to epileptic neuronal activity [28].

2. Effects of pH transients on presynaptic function

Loading of synaptic vesicles with different neurotransmitters depends on vesicular proton gradients [29]. Hence, variations in intracellular pH could directly interfere with neurotransmitter loading. It has been shown that the glutamate uptake by astrocytes is pH sensitive and provides a mechanism which can protect neurons from glutamatergic excitotoxicity due to reversed glutamate uptake under ischemic conditions [30].

The function of proteins, enzymatic activity as well as protein–protein interactions are sensitive to alterations in pH and thus changes in pH can impact on the release of synaptic vesicles, which depends on the concerted action of a complex machinery of different proteins (for review see [31]). In particular, the initial rise in the presynaptic calcium concentration mediated via voltage-gated calcium channels [32] is pH dependent, as the opening and the conductivity of presynaptic voltage-gated calcium channels strongly depend on both extracellular and intracellular pH [33]. Protons can directly bind to sensors within the pore of the channel and thereby reduce channel conductance [34,35], shield membrane-bound charges and thus shift the channel activation voltage to more positive values [36,37]. The rise in presynaptic calcium is augmented by release of calcium from intracellular stores which is mediated via inositol 1,4,5-trisphosphate and ryanodine receptors. Both receptors also show strong pH dependence [38,39]. Studies on spontaneous vesicle release by electrophysiological methods confirmed that lowering of intracellular pH in hippocampal neurons indeed results in a decreased rate of synaptic vesicle release and hence limited excitability [40,41]. Further studies are necessary to investigate if presynaptic pH modulates synapse function mainly by alterations in calcium transients or if multiple effects add up.

3. Effects of pH transients on postsynaptic function

NMDA receptors are strongly modulated by changes in extracellular pH [42,43]. An increase in extracellular pH facilitates the activation of NMDA receptors, whereas a decrease in extracellular pH inhibits ion channel function [42–44]. The transient increase in extracellular pH elicited by high-frequency stimulation of afferents in the hippocampus has been shown to be sufficient to augment NMDA-receptor responses in vitro [45]. This is most likely also relevant in vivo both in physiological and pathophysiological conditions. In contrast, kinetics and amplitudes of AMPA- and Kainate-receptors are only marginally modulated by alteration of extracellular pH [46].

Interestingly, $GABA_A$ receptor mediated currents are enlarged by low extracellular pH, whereas a high pH rather inhibits the GABA response [47–49]. $GABA_A$ receptors also conduct bicarbonate. As a consequence, GABAergic transmission can cause alterations of both intra- and extracellular pH [11]. In contrast to the direction of chloride fluxes, which can vary in dependence of the existing chloride gradients, which are set by the cation-chloride co-transporters NKCC1 and KCC2 [50–52], the existing gradients always drive HCO_3^- out of the neurons under physiological conditions. Both gradients contribute to the balance between neuronal excitation and inhibition. Only little is known about the role of pH for signaling via $GABA_B$ receptors or receptors of other neurotransmitters.

In conclusion, electrical stimulation or synchronized neuronal activity results first in an initial transient alkaline shift of the extracellular pH that is followed by a prolonged acidosis (for review see [5]). The short-lived initial increase in pH has been shown to be sufficient to augment glutamatergic excitation by activation of NMDA receptors in acute slice experiments [45] and most likely inhibits GABAergic transmission. In contrast, under conditions of sustained stimulation [53] or pathological neuronal activity [12], the following long-lasting acidosis is predicted to diminish glutamatergic neurotransmission and boost GABAergic inhibition, which was confirmed for cultured neurons [54].

This indicates that intrinsic pH transients serve as a feedback mechanism to keep the delicate balance between neuronal excitability and inhibition but also implies that neuronal and especially synaptic pH has to be tightly controlled.

4. Mechanisms to regulate synaptic pH

In general, cellular pH homeostasis is established by transport or buffering of acid equivalents. In neurons acid loading is largely established by Na^+ independent Cl^-/HCO_3^- exchangers [55], whereas Na^+/H^+ exchangers [56], Na^+-driven Cl^-/HCO_3^- exchangers and Na^+/HCO_3^- co-transporters [57] mediate acid extrusion. Another family of bicarbonate transporters, which can be distinguished from the family of SLC4 transporters [55,58], are classified as members of the SLC26 family [59], however, if at all, members of the SLC26 family of bicarbonate transporters are thought to play a minor role for neuronal pH homeostasis [60]. Neuronal pH is also affected by monocarboxylate transporters [61] but their role in the brain under physiological conditions is limited whereas they are more important in tissues with a high energy demand like in tumors [62]. Although so far no conclusive data exist that the plasma membrane calcium ATPase also plays a direct role for pH regulation, a brain-specific isoform with a predominant synaptic localization has been described [63], which may contribute to synaptic pH homeostasis [9,10].

Bicarbonate is a very important pH buffering system because it can be regulated by respiration. Carbonic anhydrases promote the interconversion of carbon dioxide and water to bicarbonate and protons, and thereby significantly contribute to the intra- and extracellular buffering capacity in the brain [64].

For a more general comprehensive review on cellular pH sensors and regulators, see [65], [5] and [66]. In the following we will mainly focus on the Na^+/H^+ exchanger NHE1, the Na^+ coupled anion-exchangers NCBE and NDCBE, and Na^+-HCO_3^- co-transporters, all mediating acid extrusion.

5. NHE1

The transmembrane Na^+-gradient is established by the Na^+/K^+ ATPase. The Na^+ gradient is then used to energize the electroneutral exchange of one extracellular sodium for one proton by Na^+/H^+ exchangers (NHE) [67]. So far 9 different isoforms of Na^+/H^+ exchangers have been identified and all of these are expressed in