Proton Mechanisms of Neurotransmission and Calcium Signalling for Impulse Initiation, Development, and Propagation

Giuliano Molinari

Funding: No specific funding was received for this work.
Potential competing interests: No potential competing interests to declare.

Abstract

Protons are gaining increasing attention as neurotransmitters due to their extraordinary abilities to rapidly transfer electrical charge, mobilize cellular calcium and modulate ion channels. How all this is possible is currently the subject of in-depth studies and discussions concerning not only neurophysiology, but also biological materials for artificial intelligence.

This review describes some biochemical mechanisms by which protons, in combination with calcium, can initiate firing in sensory neurons and transmit impulses across synapses. Furthermore, mechanisms are put forward concerning how neurotransmitters, particularly glutamate, gamma-aminobutyric acid, adenosine triphosphate and acetylcholine, are able to generate protons.

The results of the numerous experimental works taken into consideration indicate that protons can play a fundamental role both in the generation and in the transmission of the sensory nerve impulse.

Giuliano Molinari[1,a,*]

[1] *IEEE, Piscataway, NJ 08854, USA.*

[a] ORCID iD: 0000-0002-9278-5727

[*] Correspondence: giuliano.molinari@fastwebnet.it

Keywords: Ca^{2+} signalling, subcellular calcium homeostasis, signal transduction, G-protein coupled receptors. calcium-binding proteins, H^{+} ion, ATP, excitable cells, action potential, synaptic vesicles.

1. Introduction

The importance of Na^+ and K^+ ions for nerve transmission was demonstrated by eighteen years of experimental work by Hodgkin and Huxley (A. Hodgkin & Huxley, AF, 1952). Other ion, such as H^+ and Ca^{2+}, were studied less, although Hodgkin and Huxley noted the significant role of Ca^{2+} as far back as 1949 (A. L. Hodgkin, 1976) (Fig.7). Subsequent studies confirmed the fundamental role of Ca^{2+} in proper transmission (Augustine et al., 2003; Bagur & Hajnóczky, 2017; Brini et al., 2014; Clapham, 2007; Neher & Sakaba, 2008; Pozzan et al., 1994). Dysfunctions in Ca^{2+} homeostasis and abnormal Ca^{2+} concentration levels characterize the pathological states of acidosis and alkalosis. Acidosis and alkalosis are consequences of opposite, extended changes in H^+ concentration, i.e. in pH, and can cause neurodegenerative diseases (Brini et al., 2014; Verma et al., 2022; Zündorf & Reiser, 2011) and cancer (Papavassiliou & Papavassiliou, 2021; Salucci et al., 2023; Zheng et al., 2023). In fact, acidification in acidosis depletes cellular calcium stores and depleted stores release a reduced quantity of Ca^{2+} in response to stimuli. On the contrary, in alkalosis, calcium stores are overloaded and this can produce an excessive response. Only a steady-state cell with adequately full calcium stores can respond with the right release of Ca^{2+} to the stimulus, thus transducing the signal correctly. The pathological consequences of poor/excessive responses to stimuli in acidosis/alkalosis are beyond the scope of this review; here the focus is on the physiological chemical mechanisms of neurotransmission, which underlie the rapid and highly localized transient changes in H^+ and Ca^{2+} concentrations, triggered by stimuli. Unfortunately, the in vivo analytical quantification of H^+ and Ca^{2+} ions is very difficult, as they can interact with a multitude of atomic and molecular species. Moreover, fast nerve impulses can last no more than 10 ms, intracellular pH transients and calcium spikes less than 2 ms. Consequently, H^+ and Ca^{2+} ions require sophisticated instruments for their study.

The interest in H^+ ions, identified below with the current terminology as "protons", picked up after 1980 (Bevan, S & Yeats, J, 1991; Gruol et al., 1980; Krishtal & Pidoplichko, 1980) and particularly with the technical progress of the last 25 years (Barth & Corrie, 2002; Steinegger et al., 2020).

Protons are tiny ionic particles that in an aqueous environment are acidic and highly mobile, able to rapidly transfer positive charges and to temporarily modify pH, Ca^{2+} concentration, electrical potential and the protein structure, as a result activating numerous receptors. Due to these extraordinary chemical and physical properties they are used in the preparation of organic electro-conductive materials (Song et al., 2020; Yao et al., 2020) and are attracting increasing attention as neurotransmitters (Beg et al., 2008; Davies et al., 1988; Diering & Numata, 2014; J. Huang et al., 2010; Kier, 2017; Ruffin et al., 2014; Soto et al., 2018; Tombaugh & Somjen, 1996; Traynelis & Cull-Candy, 1991; Uchitel et al., 2019; Ueno et al., 1992; Willoughby & Schwiening, 2002; Zeng & Xu, 2012). Protons have been shown to have an essential role at the synaptic level (Du et al., 2014; Fillafer & Schneider, 2016; González-Inchauspe et al., 2017; Highstein et al., 2014; Uchitel et al., 2019) and it has been posited that they are responsible for conduction in axons (Kier, 2017). Some authors have also posited a significant role in the transmission and modulation of the signal in the nervous system generally (Malchow et al., 2021; Ruusuvuori & Kaila, 2014; Soto et al., 2018; Zeng et al., 2015). However, the endogenous sources of the protons have yet to be determined. There are four candidates: Na-H exchangers, V-ATPases, carbonic anhydrases and AE3 chloride-bicarbonate exchangers (Country & Jonz, 2017; Soto et al., 2018; Warren et al., 2016; Zeng & Xu, 2012), but they appear to be insufficient (Country & Jonz, 2017). Specifically, Soto and colleagues (Soto et al., 2018) rightly observe: "*A problem of classifying protons as neurotransmitters is related to the fact that its regulated release is* *always a co-release with classical neurotransmitters"*. In addition, some criticisms have been levelled against the theory of Hodgkin and Huxley; for example, it does not explain the origin of the firing of neurons (Deng, 2017). These problems could be overcome more simply if neurotransmitters and second messengers (Newton et al., 2016) were included among the possible sources of protons, given that these molecules can generate protons, i.e., new mobile charges.

The double purpose of this review is: 1) to highlight in subsection 2.3 several endogenous sources of protons, which have so far been overlooked; 2) suggest in subsections 2.4 and 2.5 some biochemical pathways for sensory impulse initiation/transmission that can be activated by protons and Ca^{2+} ions.

2. Results and Discussion

A review and critical assessment was made of the scientific publications dealing with the topic between 01.01.1943 and 31.12.2023, all available online.

2.1. Properties of protons

With an atomic mass about 23 times lower than sodium and a radius of about 0.08 nm, the proton is the smallest and most mobile ion, thanks to its diffusion coefficients, in bulk water (Silverstein, 2021). In its hexahydrate form proton has a radius of about 0.25 nm against 0.95 nm of Na^+. It diffuses faster along and across membranes than in the cytoplasm (Silverstein, 2021). The level of proton permeability across the phospholipid membrane is tightly controlled and depends on the lipids and proteins in the membrane (DeCoursey & Hosler, 2014; Endeward et al., 2014; Kratochvil et al., 2023). There are several different routes for proton permeation, via both passive and active transport. Due to different experimental conditions, the results of many existing studies are inconsistent, however, in most measurements the proton permeability was ≥ that of Na^+ (Bozdaganyan et al., 2019). Studies with weak acids on artificial vesicles revealed that protons diffuse more rapidly than other ions through lipid bilayers, mainly in the undissociated acidic form (Anderson Norris & Powell, 1992; Tivony et al., 2022). Alternatively, in living cells, protons can cross the plasma membrane much more rapidly through specific channels, such as voltage-gated proton channels (Hv1), gramicidin A channels, and mutated aquaporins (DeCoursey, 2018; DeCoursey & Hosler, 2014). Also, the existence of CO_2-permeable aquaporins has been proved, but the permeation mechanism of CO_2 through aquaporins is not yet resolved (J. Chen et al., 2023). Carbonic anhydrases, which have a fundamental role in proton generation from CO_2 in the whole organism, including brain (Ruusuvuori & Kaila, 2014), could be less available with regards to aquaporins (J. Chen et al., 2023). Besides these routes, active transporters such as pumps and exchangers can drive protons across the plasma membrane (Doyen et al., 2022; Ruusuvuori & Kaila, 2014).

The elemental charge of the proton is the same as for other individual monovalent cations, at 1.602×10^{-19} C. Anyway, protons can transport the charge much more quickly (Brünig et al., 2022; Volkov et al., 2020), via proton-hopping (Agmon et al., 2016; Silverstein, 2021). In addition to interacting with water and the three channels mentioned above, protons can modulate (King et al., 2018) a large variety of channels and receptors, such as Voltage Gated Calcium Channels

(VGCC/CaV) (Sharma et al., 2023; Simms & Zamponi, 2014), Store Operated Calcium channels (SOC) (Kraft, 2015), calcium-activated potassium channels (K_{Ca}) (Guéguinou et al., 2014; Sancho & Kyle, 2021), inward rectifier potassium channels (Kir) (Hibino et al., 2010; Ye et al., 2016), TWIK-related acid-sensitive K^+ channel (TASK) (Duprat, 1997), proton gated Acid Sensing Ion Channels (ASIC) (Rook et al., 2021; Storozhuk et al., 2021; Zeng et al., 2015),multimodal Transient Receptor Potential channels (TRP) (Cao, 2020; Kweon et al., 2015), Pannexin 1 channels (Panx1) (Whyte-Fagundes & Zoidl, 2018), G-protein Coupled Receptors (GPCR) (Sisignano et al., 2021) and P2X2 purinergic receptors (Burnstock, 2018). The interaction depends on the species, the extracellular or intracellular position of the protons, their concentration and the type of channel (de la Roche et al., 2013). Many channels, including ASIC and TRPV1, mainly trigger activation; others, such as VGCC (Almanza et al., 2008), Panx1 (Vroman et al., 2014), and TRPV5 (Fluck et al., 2022), have a control or inhibitory function. X-ray crystallography and cryo-electron microscopy have revealed the structure of many ion channels in the inactivated/open state and, in some cases, the amino acid residues involved in gating (Catterall et al., 2020). However, a knowledge of the structures of the intermediate states at the atomic level is required in order to better understand the origin of the movement of charges in the gating mechanism (Catacuzzeno & Franciolini, 2022).

2.2. The H^+/Ca^{2+} correlation

It is known that both Ca^{2+} ions and protons are ubiquitous in organisms, at concentrations that are strictly correlated (Deplazes et al., 2019; Molinari & Nervo, 2021; Swietach et al., 2013). As mentioned in the introduction, a widespread lasting increase in their concentration produces the pathological condition known as acidosis (Hamroun et al., 2020), whilst a local and temporary increase is used currently by cells as a signal, in physiological conditions (Ruusuvuori & Kaila, 2014; Soto et al., 2018; Zeng & Xu, 2012). The correlation between protons and Ca^{2+} ions is fundamental for the transmission of the signal and depends on the high degree of solubility in an acid environment of calcium-buffering molecules. In steady cells, most calcium is bound within Ca^{2+} buffers, which are either stationary or mobile (Eisner et al., 2023). When the stimulus reaches the cell membrane activating an acidifying enzyme, such as a lipase or an esterase, the enzymatic action produces protons and hence locally and temporarily lowers pH (Molinari & Nervo, 2021). The acidity quickly dissolves part of the Ca^{2+} buffers and Ca^{2+} can therefore pass into the solution, producing calcium spikes (Molinari & Nervo, 2021), of intensity and duration proportional to the quantity of protons released (Garciarena et al., 2018; S. Huang et al., 2023; OuYang, JB et al., 1994; Swietach et al., 2013). It has been calculated that in mitochondria a fall of one unit of pH produces a 100-fold increase in the concentration of Ca^{2+} (Nicholls & Chalmers, 2004). Similarly, protons produce the release of other bivalent and trivalent ions, in particular Zn^{2+} and Mg^{2+}. The intracellular increase in proton concentrations produced by esterases and lipases can transiently affect the structures of channels and pumps, by modifying their conformation and action. Clearly, the acidifying power of lipases and esterases, including phosphatases, is a very important characteristic that allows the transformation of the chemical signal into transient electrical charges and the continuation of the signal both through the release of Ca^{2+} from cellular stores and through the influx of extracellular Ca^{2+}. However, scientific publications have almost entirely ignored this characteristic. The existence in biological membranes of voltage-sensing phosphatases (VSP) that produce the opposite transformation from an electrical signal to a chemical signal (Okamura et al., 2018) may not be coincidental. This allows us to argue that protons are at the basis of the transformation of the signal from chemical to electrical and vice versa.

2.3. Endogenous sources of H^+ ions, overlooked until now

In two prior articles, we have described how protons may be generated in different cells by second messengers with the chemical structure of an ester or anhydride, such as IP_3, ATP, NAADP, cADPR, cAMP or cGMP, by the hydrolytic action of specific enzymes (Molinari, 2015; Molinari & Nervo, 2021). The hydrolysis of an ester or anhydride produces an acid, in these cases a phosphoric acid derivative, which can rapidly dissociate, releasing protons. Table 1 provides some examples of lipases and esterases and the acids they produce, which can solubilize calcium at the cellular level. Schematic representations of the reaction are available in many cases, for example for ATP (Feng, equation 5) (Feng, PX, 2017), IP_3 (Huang, Supplementary information, Fig.S1) (J. Huang et al., 2010), cAMP (Barbosa, Fig.3) (Barbosa et al., 2011) and cGMP (Rybalkin Fig.1) (Rybalkin et al., 2013). However, it is not easy to find the complete representation, because most texts inexplicably fail to mention protons. Worse yet, the names *phosphate* and *phosphoric acid* are often used interchangeably.

Table 1. Examples of lipases and esterases, as possible sources of protons and Ca^{2+} spikes

enzyme	substrate	acid product	reference
phospholipase A_2	PC	arachidonic acid	Sun (G. Y. Sun et al., 2004)
phospholipase C	PIP_2	acid IP_3	Molinari, Fig.1A (Molinari, 2015)
phospholipase D	PC	phosphatidic acid	Cazzolli (Cazzolli et al., 2006)
ecto-ATPase	ATP	ADP + acid phosphate	Kreitzer (Kreitzer et al., 2023)
phosphodiesterase	cAMP	acid AMP	Delhaye (Delhaye & Bardoni, 2021)
phosphodiesterase	cGMP	acid GMP	Delhaye (Delhaye & Bardoni, 2021)
cADPR cyclase	cADPR	acid ADPR	Young (Young & Kirkland, 2008)
VSPs	phosphoinositides	acid phosphate	Okamura (Okamura et al., 2018)
inositol 5-phosphatase	IP_3	acid phosphate	Ooms (Ooms et al., 2009)
S1P phosphatase	S1P	acid phosphate	Wollny (Wollny et al., 2017)
alkaline phosphatase	NAADP	acid phosphate	Schmid (Schmid et al., 2012)
acetylcholinesterase	ACh	acetic acid	Fillafer (Fillafer et al., 2021)

***Abbreviations**: PC, phosphatidylcholine; PIP_2, phosphatidylinositol 4,5-bisphosphate; IP_3, inositol 1,4,5-trisphosphate; ATP, adenosine 5'-triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; cADPR, cyclic adenosine diphosphate ribose; VSP, voltage-sensing phosphatase; S1P, sphingosine 1-phosphate; NAADP, nicotinic acid adenine dinucleotide phosphate; ACh, acetylcholine.*

The products of enzymatic hydrolysis, listed in the third column of Table 1, are acidic and can therefore release protons, by dissociation. The ability of an acid to generate protons and consequently Ca^{2+} spikes depends on its dissociation constant (Ka): the higher the Ka, the stronger the acid and the number of dissociated protons. Dissociation is also largely influenced by environmental pH and the pKa corresponds to the pH value at which the acid is half dissociated. Theoretically, all lipases and esterases can generate protons, but only hydrolysis that produces an acid with pKa lower than the cellular pH will substantially release protons under physiological conditions. The Drug Bank reports pKa 4.54 and 4.82 for acetic acid and arachidonic acid, respectively. The three pKas of phosphoric acid are 2.1, 7.2, and 12.3. Its partially esterified derivatives, such as phosphatidic acid and the acids produced by hydrolysis of cyclic nucleotides, have lower pKa_1 and pKa_2, since "the replacement of a phosphoric acid hydrogen by a non-acidic group leads to an increase in the acid strength" (Kumler & Eiler, 1943).

Therefore, in physiological conditions, phospholipases (i.e. PLA2, PLC, and PLD), triphosphatases (i.e. ecto-ATPase) and phosphodiesterases are acidifying enzymes, since their acid derivatives have lower pKas than the cellular pH. Numerous experimental studies support this statement. Some doubt may remain about the acidifying power of phosphomonoesterases (phosphatases) such as 5PTase, S1P phosphatase and NAADP phosphatase, due to the possible high pKa_3 value of their phosphoric derivative. However, the mechanism of phosphomonoesters hydrolysis by phosphatases proceeds through a transition state of the phosphoryl group, which involves a redistribution of the charges (De Vivo et al., 2007; Duarte et al., 2015). Accordingly, with reference to a cellular average pH=7.2, it is reasonable to assume that phosphatase hydrolysis produces the inorganic acid phosphates H2PO4- and HPO4-- in a ratio of approximately 50:50 and a resulting pH between 6.0 and 6.5. These values are sufficiently acidic for the release of bound calcium and the generation of Ca^{2+} spikes. The acidifying power of phosphatases has so far been studied in plant roots, soil microorganisms and earthworms (Moro et al., 2021; Tibbett, 2002; Vos et al., 2023) where the improvement of calcium and phosphates solubility is important for plant nutrition. For soils with pH around 6.0 a decrease in pH was shown, specifically related to phosphomonoesterase activity (Moro et al., 2021).

2.4. Pre-synaptic transmission of the impulse in sensory neurons

Protons can contribute to the generation and transmission of impulses in sensory neurons via biochemical mechanisms that differ in modality and effects (Silbering & Benton, 2010).

In the specific case of neurons sensitive to a sour taste, it has been shown in mammals that protons can directly cause firing by opening the OTOP1 channel (Chang et al., 2010; Teng et al., 2022; Tu et al., 2018).

> *"In response to acidic stimuli, the sour receptor, OTOP1, conducts protons into the cell cytosol. This changes the membrane potential directly, and the change in intracellular pH blocks KIR2.1 K+ channels, which further depolarizes the membrane potential. With sufficient depolarization, voltage-gated Na+ channels open causing a train of action potentials that open voltage-gated calcium channels and lead to neurotransmitter release" (Liman & Kinnamon, 2021).*

The pathway is more complex in the case of sensory neurons with GPCR-type metabotropic receptors at the distal termination of the axon. These are very common in mammals (Imenez Silva & Wagner, 2022; Liccardo et al., 2022) for the transmission of visual stimuli (Xue et al., 2011), nociceptive stimuli (Geppetti, P et al., 2015), odor (G. Liu et al., 2006; Szebenyi et al., 2014) and taste, limited to taste/flavour perceptions of sweet, bitter, umami and kokumi (Ahmad & Dalziel, 2020; Deshpande et al., 2010; Lee & Owyang, 2017). In these cases, the biochemical mechanism begins with the activation of a phospholipase C (PLC) (Balla, 2010; Weernink et al., 2007) which hydrolyzes the phosphatidylinositol (4,5)-bisphosphate of the neuronal membrane. The reaction for several enzyme isoforms is pH- and Ca^{2+}-dependent (Banno & Nozawa, 1987; Nakamura & Fukami, 2017; Roy et al., 1991). This means that the reaction can be acidifying and autocatalytic (Thakur et al., 2020), because the hydrolysis produces IP_3 and protons (J. Huang et al., 2010; Molinari, 2015; Randall et al., 2015), which in turn produce Ca^{2+} release (W. Chen et al., 2001; Križaj et al., 2011; Nedergaard, 1995; OuYang, JB et al., 1994; Thakur et al., 2020), hence promoting a rapid increase in enzymatic activity. The acidifying action has been confirmed experimentally at the presynaptic termination (Caldwell et al., 2013; Rossano et al., 2013; L. Zhang et al., 2016).

The increase in cytosolic Ca^{2+} concentration, induced by the direct proton influx or by the acidifying action of PLC, can have a threefold contribution:

1. Solubilization of cytosolic Ca^{2+} buffers (Molinari & Nervo, 2021; OuYang, JB et al., 1994)
2. Ca^{2+} release from endoplasmic reticulum stores (Woll & Van Petegem, 2022)
3. Ca^{2+} influx by stimulation of the SOCs (D. Wei et al., 2017)

The latter is fundamental for neurotransmission, since the influx of Ca^{2+} as well as the influx of protons can constitute the first step of depolarization.

A second step may follow rapidly with the opening of:

- low threshold VGCC/CaV channels (Dolphin, 2020; Harding & Zamponi, 2022; Ramachandran et al., 2022; Tombaugh & Somjen, 1997) permeable to Ca^{2+}
- TRP (Cao, 2020; Henrich & Buckler, 2009; J. Huang et al., 2010; Zeng & Xu, 2012) and ASIC (X. Liu et al., 2020) channels permeable to Ca^{2+} and Na^+(Hu et al., 2021).

These new influxes of Ca^{2+} and Na+ can further promote depolarization. Moreover, the increase in Ca^{2+} concentration in the cytosol modulates calcium-activated potassium channels (Hou et al., 2008; Orfali & Albanyan, 2023; Shah et al., 2022).

The above studies jointly demonstrate that protons, together with Ca^{2+} ions, can start the process of membrane depolarization not only in neurons sensitive to a sour taste, but also in many other neurons with GPCR-type receptors. It is likely that the three ions, H^+, Ca^{2+} and Na^+ contribute cooperatively (Dixon et al., 2022; Moreno et al., 2016) and to varying degrees to depolarization until the threshold value is reached.

When the threshold value is exceeded Voltage Gated Sodium Channels (NaV) open, generating the action potential (Catterall et al., 2005; A. Hodgkin & Huxley, AF, 1952). This produces the exocytosis of the vesicles and the release of the neurotransmitters into the synaptic cleft (Ge et al., 2022; Wu et al., 2014).

In the following repolarization phase the NaV channels close and the Kv (Grider et al., 2022; A. Hodgkin & Huxley, AF, 1952; Kariev & Green, 2022), K_{Ca} and Hv1 proton channels (DeCoursey, 2018; Han et al., 2022) open enabling the efflux respectively of the K^+ ions and the protons leading to the rebinding of Ca^{2+} and the return to static conditions. Pumps and exchangers contribute to the control of the entire process (Brini et al., 2014).

In the eye, the activation of GPCRs via the PLC/IP_3 pathway occurs by means of the cells containing melanopsin, whilst the cells of the retina containing rhodopsin and the cells of the auricular cochlea follow a different pathway, via PDE/cGMP (C.-K. Chen et al., 2015; Marchetta et al., 2022). In this case, the protons are generated by the hydrolysis of cGMP and the dissociation of acid glutamate, as described below in subsection 2.4. The role of protons in hair cell transmission is currently under debate (Contini et al., 2022).

In relation to the sensory neurons that transmit mechanical stimuli, it is believed that in mammals these neurons generally respond via mechanoelectrical channels (Douguet & Honoré, 2019). The physical stimulus induces the opening of ionic channels enabling the influx of Ca^{2+}, depolarization and the generation of the action potential. The mechanisms for the activation of the channels are not clear (Bavi et al., 2017). In some cases, ASIC channels (Cheng et al., 2018) or GPCR receptors (Lin et al., 2022) are involved. Moreover, it has been shown that the G protein-coupled receptor OGR1 (GPR68) responds to mechanical stimuli and to protons via the PLC/IP_3 pathway (Iliff, AJ & Xu, XZS, 2018; W.-C. Wei et al., 2018).

To sum up, for the above sensorial neurons, with ionotropic channels of the OTOP, TRP, ASIC type or metabotropic channels of the GPCR type, protons are essential to increase the cytosolic Ca^{2+} concentration. For all these cases it is therefore possible to respond to the criticisms of the Hodgkin and Huxley theory and to affirm that protons, inducing with Ca^{2+} the initial depolarization steps, via proton influx and/or proton-induced calcium influx, may be at the origin of firing.

2.5. Synaptic transmission of the impulse

Neurotransmitters include compounds, shown in Table 1, with an ester, anhydride or acid-type structure that can therefore generate protons. Below, four fundamental neurotransmitters are considered, released in the ribbon-type synapses by vesicle exocytosis: ACh, ATP, gamma-aminobutyric acid (GABA) and glutamate (Glu). ACh is an ester, ATP is a phosphoanhydride, GABA and Glu are amino acids. It is worth clarifying something regarding the latter: glutamate is the name given to a neutral salt and this can lead to confusion. In fact, for the acid strength GABA and Glu are very similar amino acids: they have respectively 4.0 and 4.3 pKa. For that reason, in vesicles where the pH is acidic (Anderson & Orci, 1988; Egashira et al., 2016; Fuldner, HH & Stadler, H, 1982; Michaelson, DM & Angel, I, 1980; Miesenbock, G & De Angelis, DA, 1998), they are both partially undissociated, in the protonate form; therefore, for the sake of coherence, like GABA, Glu should be called acid glutamate. When they are released in a neutral or slightly alkaline environment, such as the synaptic cleft in the static state, these undissociated acid molecules tend to dissociate, each in its respective anion and a proton, as shown in Table 2.

Table 2. Protonated and deprotonated states of acid neurotransmitters

VESICLE LUMEN		SYNAPTIC CLEFT
acid glutamate	⇌	glutamate$^-$ + H^+
γ-aminobutyric acid	⇌	γ-aminobutyrate$^-$ + H^+

Therefore, it is evident that vesicle exocytosis produces inter-synaptic acidification (Ahdut-Hacohen et al., 2004; DeVries, 2001; Kolen et al., 2023; Miesenbock, G & De Angelis, DA, 1998; Palmer et al., 2003; Soto et al., 2018; Uchitel et al., 2019) through the release of protons due to the acid content of vesicles and that the two acid neurotransmitters Glu and GABA may be, in glutamatergic or respectively GABAergic vesicles, the principal source of the protons. The importance of this source is shown by the fact that the organism consumes energy to recycle Glu and GABA in the vesicles sufficiently rapidly to reuse them (Eriksen et al., 2016; Marx et al., 2015; Pathak et al., 2015; Pulido, C & Ryan, TA, 2021).

ATP is an important signalling molecule (Burnstock, 2020; Dunn & Grider, 2023) as well as being a fundamental source of cellular energy, produced by mitochondria and other cellular structures (Morelli et al., 2020). Unlike Ca^{2+}, its concentration is high inside the cell and low outside. As an extracellular neurotransmitter, ATP can be released, or co-released from synaptic vesicles and activates two families of purinergic receptors, P1 and P2, for adenosine and ATP/ADP, respectively (Burnstock, 2020). The hydrolysis of ATP produces energy, ADP and acid phosphate, which in turn releases a proton. Similarly, one more step can lead to AMP. The products of hydrolysis can have a modulatory effect on retinal synapses (Kreitzer et al., 2023; Vroman et al., 2014) or, if in excess, cause inflammation and brain disorders (Di Virgilio et al., 2023; Dias et al., 2023; Vultaggio-Poma et al., 2022).

Regarding the ACh, the protons are released by the acetic acid produced by the hydrolytic split of the ester bond by the cholinesterases: acetylcholinesterase and butyryl-cholinesterase. The reaction is very rapid and produces choline and acetic acid. For a long time, it was believed that the acetic acid and choline, constituting the ACh, were neurologically inactive molecules. It is still believed that the activity of ACh concerns the entire molecule because the limited use of anticholinesterases inhibits the response in direct proportion to the inhibitor dose and the response increases with the accumulation of ACh (Malik, 1970). From this standpoint, cholinesterases have the sole function of rapidly eliminating the ACh, after its action. Today, we know that both constituents, choline and acetic acid, carry out a specific neurologically significant action (Mike, A et al., 2000; Wang et al., 2011) and that acetylcholinesterase may be indispensable for the action of ACh (Fillafer et al., 2021; Fillafer & Schneider, 2016). In addition, it has been posited that cholinergic transmission is due to the protonation of the postsynaptic membrane, caused by the acetic acid derived from the hydrolysis of ACh (Fillafer et al., 2021).

If the hypothesis that ACh can also act via its constituents were confirmed, it would be easier to clarify a number of questions that have been perplexing for some time. In addition, the fact that the four neurotransmitters ATP, ACh, Glu and GABA can release protons explains the observation of Soto et al. regarding co-release, as cited in the introduction.

The protons released by Glu, GABA, ATP or ACh acidify the inter-synaptic space and can activate acid-sensitive receptors at the postsynaptic termination together with specific receptors for Glu, GABA, ATP and ACh. There are numerous proton-sensitive receptors in the postsynaptic termination (Holzer, 2011), both ionotropic such as ASICs (Cheng et al., 2018; Rook et al., 2021), TRPV1 (Kweon et al., 2015; Leffler, A et al., 2006; Ryu et al., 2007; Semtner et al., 2007), CaV3 (Lipkin et al., 2021) and metabotropic, of the TASK type (Fan et al., 2022) and GPCRs (Sisignano et al., 2021), The proton activation of the postsynaptic receptor can foster the opening of ionic channels (Boillat, A et al., 2014; Henrich & Buckler, 2009), depolarization and the generation of a new action potential, enabling the impulse to continue (Burke & Bender, 2019; Fillafer et al., 2021; Highstein et al., 2014).

Furthermore, many ligand receptors, specific for Glu, GABA and ACh, of the GPCR type, such as Group1 Glu (Suh et al., 2018; Y.-G. Sun et al., 2016), GABAb (Negri et al., 2022), nicotinic α7 (King et al., 2018; Papke, RI & Lindstrom, JM, 2020) and muscarinic M1, M3 and M5 (Brown, 2019; Sam & Bordoni, 2022) receptors are activated by protons generated by PLCs. Ionotropic GABAa are also activated by the PLCs (Nicholson et al., 2018). On the contrary, most ionotropic postsynaptic receptors of glutamate are inhibited by the protons, particularly AMPARs (Ihle, Eva C. & Patneau, Doris K., 2000), Kainate receptors (Mott et al., 2003) and NMDARs (Dravid et al., 2007; J.-B. Zhang et al., 2018).

It is evident that protons may act at the synaptic level in various ways and via a large number of receptors. However, since they are highly mobile and reactive but have low specificity, it is logical to attribute to protons mainly the quantitative aspects of the mechanisms of neurotransmission. Whilst the qualitative aspects could be modulated by variations in the frequency, intensity and duration of the proton impulse, by a parallel series of events such as variations in the concentration of other ions, the type of other neurotransmitters involved, the receptors activated, their interrelations and their responses. In line with the general principle of co-release and co-transmission (Hunt et al., 2022; Svensson et al., 2019).

3. Conclusions

Subsection 2.2 of the discussion points out the interdependence of protons and Ca^{2+} ions due to their chemical properties and it is useful to bear this in mind when studying the role of these ions in neurotransmission. The following subsections cite numerous experimental works the result of which, when taken together, provide an answer to the double aim of this paper and support the hypothesis that protons, with Ca^{2+} ions, may play a fundamental role both in the generation and the biochemical transmission of the nerve impulse. The protons could be the basis of the transformation of chemical signals into electrical signals and vice versa in the nervous system. Specifically, subsection 2.3 lists in Table 1 some important enzymatic proton sources for cell signalling. Subsection 2.4 describes how protons are able to trigger the depolarization of sensorial neurons by directly opening ionotropic channels or activating GPCR receptors, via PLC/IP_3 and the mobilization of Ca^{2+}, thereby contributing to the generation of the action potential and the exocytosis of the vesicles. Subsection 2.5 describes the mechanisms by which neurotransmitters in the vesicles, such as Glu, GABA, ATP and ACh, are able to become the sources of protons, generating them and, via the protons, fostering the transmission of the impulse through the synaptic cleft to the postsynaptic termination and beyond. To conclude, the role of protons in neurotransmission may be more important than has so far been believed and could lead to many surprising and important discoveries in the future.

Acknowledgements

I would like to express my lasting gratitude to Henrique Soto, Instituto de Fisiologia, BUAP, Puebla, Todd P. Silverstein, Department of Chemistry, Willamette University, Salem, Oregon and Richard D. Rabbitt, Department of Biomedical Engineering, University of Utah, Salt Lake City for reading the manuscript and for their helpful and valuable suggestions.

References

- Agmon, N., Bakker, H. J., Campen, R. K., Henchman, R. H., Pohl, P., Roke, S., Thämer, M., & Hassanali, A. (2016). Protons and Hydroxide Ions in Aqueous Systems. *Chemical Reviews*, *116*(13), 7642–7672. https://doi.org/10.1021/acs.chemrev.5b00736
- Ahdut-Hacohen, R., Duridanova, D., Meiri, H., & Rahamimoff, R. (2004). Hydrogen ions control synaptic vesicle ion channel activity in *Torpedo* electromotor neurones: H^+ dependence of synaptic vesicle ion channels. *The Journal of Physiology*, *556*(2), 347–352. https://doi.org/10.1113/jphysiol.2003.058818
- Ahmad, R., & Dalziel, J. E. (2020). G Protein-Coupled Receptors in Taste Physiology and Pharmacology. *Frontiers in Pharmacology*, *11*, 587664. https://doi.org/10.3389/fphar.2020.587664
- Almanza, A., Mercado, F., Vega, R., & Soto, E. (2008). Extracellular pH modulates the voltage-dependent Ca2+ current and low threshold K+ current in hair cells. *Neurochemical Research*, *33*(8), 1435–1441. https://doi.org/10.1007/s11064-007-9565-9
- Anderson Norris, F., & Powell, G. L. (1992). Characterization of CO2/carbonic acid mediated proton flux through phosphatidylcholine vesicles as model membranes. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1111*(1), 17–26. https://doi.org/10.1016/0005-2736(92)90269-R
- Anderson, R. G., & Orci, L. (1988). A view of acidic intracellular compartments. *Journal of Cell Biology*, *106*(3), 539–543. https://doi.org/10.1083/jcb.106.3.539
- Augustine, G. J., Santamaria, F., & Tanaka, K. (2003). Local Calcium Signaling in Neurons. *Neuron*, *40*(2), 331–346. https://doi.org/10.1016/S0896-6273(03)00639-1
- Bagur, R., & Hajnóczky, G. (2017). Intracellular Ca2+ Sensing: Its Role in Calcium Homeostasis and Signaling. *Molecular Cell*, *66*(6), 780–788. https://doi.org/10.1016/j.molcel.2017.05.028
- Balla, T. (2010). Putting G protein–coupled receptor-mediated activation of phospholipase C in the limelight. *Journal of General Physiology*, *135*(2), 77–80. https://doi.org/10.1085/jgp.200910396
- Banno, Y., & Nozawa, Y. (1987). Characterization of partially purified phospholipase C from human platelet membranes. *Biochemical Journal*, *248*(1), 95–101. https://doi.org/10.1042/bj2480095
- Barbosa, M. L. de C., Fumian, M. M., Miranda, A. L. P. de, Barreiro, E. J., & Lima, L. M. (2011). Therapeutic approaches for tumor necrosis factor inhibition. *Brazilian Journal of Pharmaceutical Sciences*, *47*(3), 427–446.

https://doi.org/10.1590/S1984-82502011000300002

- Barth, A., & Corrie, J. E. T. (2002). Characterization of a New Caged Proton Capable of Inducing Large pH Jumps. *Biophysical Journal*, *83*(5), 2864–2871. https://doi.org/10.1016/S0006-3495(02)75295-8
- Bavi, N., Nikolaev, Y. A., Bavi, O., Ridone, P., Martinac, A. D., Nakayama, Y., Cox, C. D., & Martinac, B. (2017). Principles of Mechanosensing at the Membrane Interface. In R. M. Epand & J.-M. Ruysschaert (A c. Di), *The Biophysics of Cell Membranes* (Vol. 19, pp. 85–119). Springer Singapore.https://doi.org/10.1007/978-981-10-6244-5_4
- Beg, A. A., Ernstrom, G. G., Nix, P., Davis, M. W., & Jorgensen, E. M. (2008). Protons Act as a Transmitter for Muscle Contraction in C. elegans. *Cell*, *132*(1), 149–160. https://doi.org/10.1016/j.cell.2007.10.058
- Bevan, S & Yeats, J. (1991). Protons activate a cation conductance in a sub-population of rat dorsal root ganglion neurones. *The Journal of Physiology*, *433*, 145–161. https://doi.org/10.1113/jphysiol.1991.sp018419
- Boillat, A, Alijevic, O, & Kellenberger, S. (2014). Calcium entry via TRPV1 but not ASICs induces neuropeptide release from sensory neurons. *Mol Cell Neurosci*, *61*, 13–22. https://doi.org/10.1016/j.mcn.2014.04.007
- Bozdaganyan, M. E., Lokhmatikov, A. V., Voskoboynikova, N., Cherepanov, D. A., Steinhoff, H.-J., Shaitan, K. V., & Mulkidjanian, A. Y. (2019). Proton leakage across lipid bilayers: Oxygen atoms of phospholipid ester linkers align water molecules into transmembrane water wires. *Biochimica et Biophysica Acta (BBA) - Bioenergetics*, *1860*(6), 439–451. https://doi.org/10.1016/j.bbabio.2019.03.001
- Brini, M., Calì, T., Ottolini, D., & Carafoli, E. (2014). Neuronal calcium signaling: Function and dysfunction.*Cellular and Molecular Life Sciences*, *71*(15), 2787–2814. https://doi.org/10.1007/s00018-013-1550-7
- Brown, D. A. (2019). Acetylcholine and cholinergic receptors.*Brain and Neuroscience Advances*, *3*, 239821281882050. https://doi.org/10.1177/2398212818820506
- Brünig, F. N., Rammler, M., Adams, E. M., Havenith, M., & Netz, R. R. (2022). Spectral signatures of excess-proton waiting and transfer-path dynamics in aqueous hydrochloric acid solutions. *Nature Communications*, *13*(1), 4210. https://doi.org/10.1038/s41467-022-31700-x
- Burke, K. J., & Bender, K. J. (2019). Modulation of Ion Channels in the Axon: Mechanisms and Function*Frontiers in Cellular Neuroscience*, *13*, 221. https://doi.org/10.3389/fncel.2019.00221
- Burnstock, G. (2018). Purine and purinergic receptors. *Brain and Neuroscience Advances*, *2*, 239821281881749. https://doi.org/10.1177/2398212818817494
- Burnstock, G. (2020). Introduction to Purinergic Signaling. In P. Pelegrín (A c. Di),*Purinergic Signaling* (Vol. 2041, pp. 1–15). Springer New York. https://doi.org/10.1007/978-1-4939-9717-6_1
- Caldwell, L., Harries, P., Sydlik, S., & Schwiening, C. J. (2013). Presynaptic pH and vesicle fusion in*Drosophila* larvae neurones. *Synapse*, *67*(11), 729–740. https://doi.org/10.1002/syn.21678
- Cao, E. (2020). Structural mechanisms of transient receptor potential ion channels.*Journal of General Physiology*, *152*(3), e201811998. https://doi.org/10.1085/jgp.201811998
- Catacuzzeno, L., & Franciolini, F. (2022). The 70-year search for the voltage-sensing mechanism of ion channels.*The Journal of Physiology*, *600*(14), 3227–3247. https://doi.org/10.1113/JP282780
- Catterall, W. A., Goldin, A. L., & Waxman, S. G. (2005). International Union of Pharmacology. XLVII. Nomenclature and

Structure-Function Relationships of Voltage-Gated Sodium Channels. *Pharmacological Reviews*, *57*(4), 397–409. https://doi.org/10.1124/pr.57.4.4

- Catterall, W. A., Lenaeus, M. J., & Gamal El-Din, T. M. (2020). Structure and Pharmacology of Voltage-Gated Sodium and Calcium Channels. *Annual Review of Pharmacology and Toxicology*, *60*(1), 133–154. https://doi.org/10.1146/annurev-pharmtox-010818-021757
- Cazzolli, R., Shemon, A., Fang, M., & Hughes, W. (2006). Phospholipid signalling through phospholipase D and phosphatidic acid. *IUBMB Life (International Union of Biochemistry and Molecular Biology: Life)*, *58*(8), 457–461. https://doi.org/10.1080/15216540600871142
- Chang, R. B., Waters, H., & Liman, E. R. (2010). A proton current drives action potentials in genetically identified sour taste cells. *Proceedings of the National Academy of Sciences*, *107*(51), 22320–22325. https://doi.org/10.1073/pnas.1013664107
- Chen, C.-K., Woodruff, M. L., & Fain, G. L. (2015). Rhodopsin kinase and recoverin modulate phosphodiesterase during mouse photoreceptor light adaptation. *Journal of General Physiology*, *145*(3), 213–224. https://doi.org/10.1085/jgp.201411273
- Chen, J., Yue, K., Shen, L., Zheng, C., Zhu, Y., Han, K., & Kai, L. (2023). Aquaporins and CO2 diffusion across biological membrane. *Frontiers in Physiology*, *14*, 1205290. https://doi.org/10.3389/fphys.2023.1205290
- Chen, W., Chen, C., Yang, K., Chang, W., Su, M., Wu, C., & Wu, M. (2001). Arachidonic acid-induced H^+ and Ca^{2+} increases in both the cytoplasm and nucleoplasm of rat cerebellar granule cells. *The Journal of Physiology*, *537*(2), 497–510. https://doi.org/10.1111/j.1469-7793.2001.00497.x
- Cheng, Y.-R., Jiang, B.-Y., & Chen, C.-C. (2018). Acid-sensing ion channels: Dual function proteins for chemo-sensing and mechano-sensing. *Journal of Biomedical Science*, *25*(1), 46. https://doi.org/10.1186/s12929-018-0448-y
- Clapham, D. E. (2007). Calcium Signaling. *Cell*, *131*(6), 1047–1058. https://doi.org/10.1016/j.cell.2007.11.028
- Contini, D., Holstein, G. R., & Art, J. J. (2022). Simultaneous Dual Recordings From Vestibular Hair Cells and Their Calyx Afferents Demonstrate Multiple Modes of Transmission at These Specialized Endings. *Frontiers in Neurology*, *13*, 891536. https://doi.org/10.3389/fneur.2022.891536
- Country, M. W., & Jonz, M. G. (2017). Calcium dynamics and regulation in horizontal cells of the vertebrate retina: Lessons from teleosts. *Journal of Neurophysiology*, *117*(2), 523–536. https://doi.org/10.1152/jn.00585.2016
- Davies, N. W., Lux, H. D., & Morad, M. (1988). Site and mechanism of activation of proton-induced sodium current in chick dorsal root ganglion neurones. *The Journal of Physiology*, *400*(1), 159–187. https://doi.org/10.1113/jphysiol.1988.sp017116
- de la Roche, J., Eberhardt, M. J., Klinger, A. B., Stanslowsky, N., Wegner, F., Koppert, W., Reeh, P. W., Lampert, A., Fischer, M. J. M., & Leffler, A. (2013). The Molecular Basis for Species-specific Activation of Human TRPA1 Protein by Protons Involves Poorly Conserved Residues within Transmembrane Domains 5 and 6. *Journal of Biological Chemistry*, *288*(28), 20280–20292. https://doi.org/10.1074/jbc.M113.479337
- De Vivo, M., Ensing, B., Dal Peraro, M., Gomez, G. A., Christianson, D. W., & Klein, M. L. (2007). Proton Shuttles and Phosphatase Activity in Soluble Epoxide Hydrolase. *Journal of the American Chemical Society*, *129*(2), 387–394. https://doi.org/10.1021/ja066150c

- DeCoursey, T. E. (2018). Voltage and pH sensing by the voltage-gated proton channel, H_V 1. *Journal of The Royal Society Interface*, *15*(141), 20180108. https://doi.org/10.1098/rsif.2018.0108
- DeCoursey, T. E., & Hosler, J. (2014). Philosophy of voltage-gated proton channels.*Journal of The Royal Society Interface*, *11*(92), 20130799. https://doi.org/10.1098/rsif.2013.0799
- Delhaye, S., & Bardoni, B. (2021). Role of phosphodiesterases in the pathophysiology of neurodevelopmental disorders. *Molecular Psychiatry*, *26*(9), 4570–4582. https://doi.org/10.1038/s41380-020-00997-9
- Deng, B. (2017). Alternative Models to Hodgkin-Huxley Equations. *Bulletin of Mathematical Biology*, *79*(6), 1390–1411. https://doi.org/10.1007/s11538-017-0289-y
- Deplazes, E., White, J., Murphy, C., Cranfield, C. G., & Garcia, A. (2019). Competing for the same space: Protons and alkali ions at the interface of phospholipid bilayers. *Biophysical Reviews*, *11*(3), 483–490. https://doi.org/10.1007/s12551-019-00541-2
- Deshpande, D. A., Wang, W. C. H., McIlmoyle, E. L., Robinett, K. S., Schillinger, R. M., An, S. S., Sham, J. S. K., & Liggett, S. B. (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. *Nature Medicine*, *16*(11), 1299–1304. https://doi.org/10.1038/nm.2237
- DeVries, S. H. (2001). Exocytosed Protons Feedback to Suppress the Ca2+ Current in Mammalian Cone Photoreceptors. *Neuron*, *32*(6), 1107–1117. https://doi.org/10.1016/S0896-6273(01)00535-9
- Di Virgilio, F., Vultaggio-Poma, V., Falzoni, S., & Giuliani, A. L. (2023). Extracellular ATP: A powerful inflammatory mediator in the central nervous system. *Neuropharmacology*, *224*, 109333. https://doi.org/10.1016/j.neuropharm.2022.109333
- Dias, L., Pochmann, D., Lemos, C., Silva, H. B., Real, J. I., Gonçalves, F. Q., Rial, D., Gonçalves, N., Simões, A. P., Ferreira, S. G., Agostinho, P., Cunha, R. A., & Tomé, A. R. (2023). Increased Synaptic ATP Release and CD73-Mediated Formation of Extracellular Adenosine in the Control of Behavioral and Electrophysiological Modifications Caused by Chronic Stress. *ACS Chemical Neuroscience*, *14*(7), 1299–1309. https://doi.org/10.1021/acschemneuro.2c00810
- Diering, G. H., & Numata, M. (2014). Endosomal pH in neuronal signaling and synaptic transmission: Role of Na+/H+ exchanger NHE5. *Frontiers in Physiology*, *4*. https://doi.org/10.3389/fphys.2013.00412
- Dixon, R. E., Navedo, M. F., Binder, M. D., & Santana, L. F. (2022). Mechanisms and physiological implications of cooperative gating of clustered ion channels. *Physiological Reviews*, *102*(3), 1159–1210. https://doi.org/10.1152/physrev.00022.2021
- Dolphin, A. C. (2020). Functions of Presynaptic Voltage-gated Calcium Channels. *Function*, *2*(1), zqaa027. https://doi.org/10.1093/function/zqaa027
- Douguet, D., & Honoré, E. (2019). Mammalian Mechanoelectrical Transduction: Structure and Function of Force-Gated Ion Channels. *Cell*, *179*(2), 340–354. https://doi.org/10.1016/j.cell.2019.08.049
- Doyen, D., Poët, M., Jarretou, G., Pisani, D. F., Tauc, M., Cougnon, M., Argentina, M., Bouret, Y., & Counillon, L. (2022). Intracellular pH Control by Membrane Transport in Mammalian Cells. Insights Into the Selective Advantages of Functional Redundancy. *Frontiers in Molecular Biosciences*, *9*, 825028. https://doi.org/10.3389/fmolb.2022.825028
- Dravid, S. M., Erreger, K., Yuan, H., Nicholson, K., Le, P., Lyuboslavsky, P., Almonte, A., Murray, E., Mosley, C.,

Barber, J., French, A., Balster, R., Murray, T. F., & Traynelis, S. F. (2007). Subunit-specific mechanisms and proton sensitivity of NMDA receptor channel block: Proton sensitivity of NMDA receptor channel blockers. *The Journal of Physiology*, *581*(1), 107–128. https://doi.org/10.1113/jphysiol.2006.124958

- Du, J., Reznikov, L. R., Price, M. P., Zha, X., Lu, Y., Moninger, T. O., Wemmie, J. A., & Welsh, M. J. (2014). Protons are a neurotransmitter that regulates synaptic plasticity in the lateral amygdala. *Proceedings of the National Academy of Sciences*, *111*(24), 8961–8966. https://doi.org/10.1073/pnas.1407018111
- Duarte, F., Åqvist, J., Williams, N. H., & Kamerlin, S. C. L. (2015). Resolving Apparent Conflicts between Theoretical and Experimental Models of Phosphate Monoester Hydrolysis. *Journal of the American Chemical Society*, *137*(3), 1081–1093. https://doi.org/10.1021/ja5082712
- Dunn, J., & Grider, M. H. (2023). Physiology, Adenosine Triphosphate. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK553175/
- Duprat, F. (1997). TASK, a human background K+ channel to sense external pH variations near physiological pH. *The EMBO Journal*, *16*(17), 5464–5471. https://doi.org/10.1093/emboj/16.17.5464
- Egashira, Y., Takase, M., Watanabe, S., Ishida, J., Fukamizu, A., Kaneko, R., Yanagawa, Y., & Takamori, S. (2016). Unique pH dynamics in GABAergic synaptic vesicles illuminates the mechanism and kinetics of GABA loading. *Proceedings of the National Academy of Sciences*, *113*(38), 10702–10707. https://doi.org/10.1073/pnas.1604527113
- Eisner, D., Neher, E., Taschenberger, H., & Smith, G. (2023). Physiology of intracellular calcium buffering. *Physiological Reviews*, *103*(4), 2767–2845. https://doi.org/10.1152/physrev.00042.2022
- Endeward, V., Al-Samir, S., Itel, F., & Gros, G. (2014). How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods. *Frontiers in Physiology*, *4*. https://doi.org/10.3389/fphys.2013.00382
- Eriksen, J., Chang, R., McGregor, M., Silm, K., Suzuki, T., & Edwards, R. H. (2016). Protons Regulate Vesicular Glutamate Transporters through an Allosteric Mechanism. *Neuron*, *90*(4), 768–780. https://doi.org/10.1016/j.neuron.2016.03.026
- Fan, X., Lu, Y., Du, G., & Liu, J. (2022). Advances in the Understanding of Two-Pore Domain TASK Potassium Channels and Their Potential as Therapeutic Targets. *Molecules*, *27*(23), 8296. https://doi.org/10.3390/molecules27238296
- Feng, PX. (2017). The Mechanism of Hydrolysis Reaction of Adenosine Triphosphate Molecules for the Generation of Bio-Energy and its Properties in the Living Systems. *International Journal of Pharmaceutica Analytica Acta*, *1*(1), 001–008.
- Fillafer, C., Koll, Y. S., & Schneider, M. F. (2021). Lipid Membrane State Change by Catalytic Protonation and the Implications for Synaptic Transmission. *Membranes*, *12*(1), 5. https://doi.org/10.3390/membranes12010005
- Fillafer, C., & Schneider, M. F. (2016). On the excitation of action potentials by protons and its potential implications for cholinergic transmission. *Protoplasma*, *253*(2), 357–365. https://doi.org/10.1007/s00709-015-0815-4
- Fluck, E. C., Yazici, A. T., Rohacs, T., & Moiseenkova-Bell, V. Y. (2022). Structural basis of TRPV5 regulation by physiological and pathophysiological modulators. *Cell Reports*, *39*(4), 110737. https://doi.org/10.1016/j.celrep.2022.110737
- Fuldner, HH & Stadler, H. (1982). 31P-NMR Analysis of Synaptic Vesicles. *European Journal of Biochemistry*, *121*,

519–524.

- Garciarena, C. D., Malik, A., Swietach, P., Moreno, A. P., & Vaughan-Jones, R. D. (2018). Distinct moieties underlie biphasic H^+ gating of connexin43 channels, producing a pH optimum for intercellular communication. *The FASEB Journal*, *32*(4), 1969–1981. https://doi.org/10.1096/fj.201700876R
- Ge, L., Shin, W., Arpino, G., Wei, L., Chan, C. Y., Bleck, C. K. E., Zhao, W., & Wu, L.-G. (2022). Sequential compound fusion and kiss-and-run mediate exo- and endocytosis in excitable cells. *Science Advances*, *8*(24), eabm6049. https://doi.org/10.1126/sciadv.abm6049
- Geppetti, P, Veldhuis, NA, Lieu, TM, & Bunnett, NW. (2015). G Protein-Coupled Receptors: Dynamic Machines for Signaling Pain and Itch. *Neuron*, *88*(4), 635–649. https://doi.org/10.1016/j.neuron.2015.11.001
- González-Inchauspe, C., Urbano, F. J., Di Guilmi, M. N., & Uchitel, O. D. (2017). Acid-Sensing Ion Channels Activated by Evoked Released Protons Modulate Synaptic Transmission at the Mouse Calyx of Held Synapse. *The Journal of Neuroscience*, *37*(10), 2589–2599. https://doi.org/10.1523/JNEUROSCI.2566-16.2017
- Grider, M. H., Jessu, R., & Kabir, R. (2022). *Physiology, Action Potential: Vol. In: StatPearls [internet]. Treasure Island (FL):* StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK538143/
- Gruol, D., Barker, J., Huang, L., McDonald, J., & Smith, T. Jr. (1980). *Hydrogen ions have multiple effects on the excitability of cultured mammalian neurons*. *183*(1), 247–252. https://doi.org/10.1016/0006-8993(80)90138-9
- Guéguinou, M., Chantôme, A., Fromont, G., Bougnoux, P., Vandier, C., & Potier-Cartereau, M. (2014). KCa and Ca2+ channels: The complex thought. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1843*(10), 2322–2333. https://doi.org/10.1016/j.bbamcr.2014.02.019
- Hamroun, A., Pekar, J.-D., Lionet, A., Ghulam, A., Maboudou, P., Mercier, A., Brousseau, T., Grzych, G., & Glowacki, F. (2020). Ionized calcium: Analytical challenges and clinical relevance. *Journal of Laboratory and Precision Medicine*, *5*, 22–22. https://doi.org/10.21037/jlpm-20-60
- Han, S., Peng, S., Vance, J., Tran, K., Do, N., Bui, N., Gui, Z., & Wang, S. (2022). Structural dynamics determine voltage and pH gating in human voltage-gated proton channel. *eLife*, *11*, e73093. https://doi.org/10.7554/eLife.73093
- Harding, E. K., & Zamponi, G. W. (2022). Central and peripheral contributions of T-type calcium channels in pain. *Molecular Brain*, *15*(1), 39. https://doi.org/10.1186/s13041-022-00923-w
- Henrich, M., & Buckler, K. J. (2009). Acid-evoked Ca2+ signalling in rat sensory neurones: Effects of anoxia and aglycaemia. *Pflügers Archiv - European Journal of Physiology*, *459*(1), 159–181. https://doi.org/10.1007/s00424-009-0715-6
- Hibino, H., Inanobe, A., Furutani, K., Murakami, S., Findlay, I., & Kurachi, Y. (2010). Inwardly Rectifying Potassium Channels: Their Structure, Function, and Physiological Roles. *Physiological Reviews*, *90*(1), 291–366. https://doi.org/10.1152/physrev.00021.2009
- Highstein, S. M., Holstein, G. R., Mann, M. A., & Rabbitt, R. D. (2014). Evidence that protons act as neurotransmitters at vestibular hair cell–calyx afferent synapses. *Proceedings of the National Academy of Sciences*, *111*(14), 5421–5426. https://doi.org/10.1073/pnas.1319561111
- Hodgkin, A., & Huxley, AF, A. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. *The Journal of Physiology*, *117*(4), 500–544. https://doi.org/10.1113/jphysiol.1952.sp004764

- Hodgkin, A. L. (1976). Chance and design in electrophysiology: An informal account of certain experiments on nerve carried out between 1934 and 1952. *The Journal of Physiology*, *263*(1), 1–21. https://doi.org/10.1113/jphysiol.1976.sp011620
- Holzer, P. (2011). Acid sensing by visceral afferent neurones: Acid sensing by visceral afferent neurones.*Acta Physiologica*, *201*(1), 63–75. https://doi.org/10.1111/j.1748-1716.2010.02143.x
- Hou, S., Xu, R., Heinemann, S. H., & Hoshi, T. (2008). Reciprocal regulation of the Ca2+ and H+ sensitivity in the SLO1 BK channel conferred by the RCK1 domain. *Nature Structural & Molecular Biology*, *15*(4), 403–410. https://doi.org/10.1038/nsmb.1398
- Hu, F., Song, X., & Long, D. (2021). Transient receptor potential ankyrin 1 and calcium: Interactions and association with disease (Review). *Experimental and Therapeutic Medicine*, *22*(6), 1462. https://doi.org/10.3892/etm.2021.10897
- Huang, J., Liu, C.-H., Hughes, S. A., Postma, M., Schwiening, C. J., & Hardie, R. C. (2010). Activation of TRP Channels by Protons and Phosphoinositide Depletion in Drosophila Photoreceptors. *Current Biology*, *20*(3), 189–197. https://doi.org/10.1016/j.cub.2009.12.019
- Huang, S., Shen, L., Roelfsema, M. R. G., Becker, D., & Hedrich, R. (2023). Light-gated channelrhodopsin sparks proton-induced calcium release in guard cells. *Science*, *382*(6676), 1314–1318. https://doi.org/10.1126/science.adj9696
- Hunt, P. J., Kochukov, M., Pekarek, B. T., Belfort, B. D. W., Romero, J. M., Swanson, J. L., & Arenkiel, B. R. (2022). Co-transmitting neurons in the lateral septal nucleus exhibit features of neurotransmitter switching. *IBRO Neuroscience Reports*, *12*, 390–398. https://doi.org/10.1016/j.ibneur.2022.05.003
- Ihle, Eva C. & Patneau, Doris K. (2000). Modulation of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid Receptor Desensitization by Extracellular Protons. *Molecular Pharmacology*, *58*(6), 1204–1212. https://doi.org/10.1124/mol.58.6.1204
- Iliff, AJ & Xu, XZS. (2018). A Mechanosensitive GPCR that Detects the Bloody Force.*Cell*, *173*(3), 542–544. https://doi.org/10.1016/j.cell.2018.04.001
- Imenez Silva, P. H., & Wagner, C. A. (2022). Physiological relevance of proton-activated GPCRs.*Pflügers Archiv - European Journal of Physiology*, *474*(5), 487–504. https://doi.org/10.1007/s00424-022-02671-1
- Kariev, A. M., & Green, M. E. (2022). Protons in Gating the Kv1.2 Channel: A Calculated Set of Protonation States in Response to Polarization/Depolarization of the Channel, with the Complete Proposed Proton Path from Voltage Sensing Domain to Gate. *Membranes*, *12*(7), 718. https://doi.org/10.3390/membranes12070718
- Kier, L. (2017). Nerve Conduction Through Dendrites via Proton Hopping.*Current Computer Aided-Drug Design*, *13*(1), 57–59. https://doi.org/10.2174/1573409912666160725113233
- King, J. R., Ullah, A., Bak, E., Jafri, M. S., & Kabbani, N. (2018). Ionotropic and Metabotropic Mechanisms of Allosteric Modulation of α7 Nicotinic Receptor Intracellular Calcium.*Molecular Pharmacology*, *93*(6), 601–611. https://doi.org/10.1124/mol.117.111401
- Kolen, B., Borghans, B., Kortzak, D., Lugo, V., Hannack, C., Guzman, R. E., Ullah, G., & Fahlke, C. (2023). Vesicular glutamate transporters are H+-anion exchangers that operate at variable stoichiometry. *Nature Communications*, *14*(1), 2723. https://doi.org/10.1038/s41467-023-38340-9

- Kraft, R. (2015). STIM and ORAI proteins in the nervous system. *Channels*, *9*(5), 245–252. https://doi.org/10.1080/19336950.2015.1071747
- Kratochvil, H. T., Watkins, L. C., Mravic, M., Thomaston, J. L., Nicoludis, J. M., Somberg, N. H., Liu, L., Hong, M., Voth, G. A., & DeGrado, W. F. (2023). Transient water wires mediate selective proton transport in designed channel proteins. *Nature Chemistry*, *15*(7), 1012–1021. https://doi.org/10.1038/s41557-023-01210-4
- Kreitzer, M. A., Vredeveld, M., Tinner, K., Powell, A. M., Schantz, A. W., Leininger, R., Merillat, R., Gongwer, M. W., Tchernookova, B. K., & Malchow, R. P. (2023). ATP-mediated Increase in H^+ Efflux from Retinal Müller Cells of the Axolotl. *Journal of Neurophysiology*, jn.00321.2023. https://doi.org/10.1152/jn.00321.2023
- Krishtal, O., & Pidoplichko, V. (1980). A receptor for protons in the nerve cell membrane. *Neuroscience*, *5*(12), 2325–2327. https://doi.org/10.1016/0306-4522(80)90149-9
- Križaj, D., Mercer, A. J., Thoreson, W. B., & Barabas, P. (2011). Intracellular pH modulates inner segment calcium homeostasis in vertebrate photoreceptors. *American Journal of Physiology-Cell Physiology*, *300*(1), C187–C197. https://doi.org/10.1152/ajpcell.00264.2010
- Kumler, W. D., & Eiler, J. J. (1943). The Acid Strength of Mono and Diesters of Phosphoric Acid. The n-Alkyl Esters from Methyl to Butyl, the Esters of Biological Importance, and the Natural Guanidine Phosphoric Acids. *Journal of the American Chemical Society*, *65*(12), 2355–2361. https://doi.org/10.1021/ja01252a028
- Kweon, H.-J., Yu, S.-Y., Kim, D.-I., & Suh, B.-C. (2015). Differential Regulation of Proton-Sensitive Ion Channels by Phospholipids: A Comparative Study between ASICs and TRPV1. *PLOS ONE*, *10*(3), e0122014. https://doi.org/10.1371/journal.pone.0122014
- Lee, A., & Owyang, C. (2017). Sugars, Sweet Taste Receptors, and Brain Responses. *Nutrients*, *9*(7), 653. https://doi.org/10.3390/nu9070653
- Leffler, A, Mönter, B, & Koltzenburg, M. (2006). The role of the capsaicin receptor TRPV1 and acid-sensing ion channels (ASICS) in proton sensitivity of subpopulations of primary nociceptive neurons in rats and mice. *Neuroscience*, *139*(2), 699–709. https://doi.org/10.1016/j.neuroscience.2005.12.020
- Liccardo, F., Luini, A., & Di Martino, R. (2022). Endomembrane-Based Signaling by GPCRs and G-Proteins. *Cells*, *11*(3), 528. https://doi.org/10.3390/cells11030528
- Liman, E. R., & Kinnamon, S. C. (2021). Sour taste: Receptors, cells and circuits. *Current Opinion in Physiology*, *20*, 8–15. https://doi.org/10.1016/j.cophys.2020.12.006
- Lin, H.-H., Ng, K.-F., Chen, T.-C., & Tseng, W.-Y. (2022). Ligands and Beyond: Mechanosensitive Adhesion GPCRs. *Pharmaceuticals*, *15*(2), 219. https://doi.org/10.3390/ph15020219
- Lipkin, A. M., Cunniff, M. M., Spratt, P. W. E., Lemke, S. M., & Bender, K. J. (2021). Functional Microstructure of Ca_V-Mediated Calcium Signaling in the Axon Initial Segment. *The Journal of Neuroscience*, *41*(17), 3764–3776. https://doi.org/10.1523/JNEUROSCI.2843-20.2021
- Liu, G., Badeau, R. M., Tanimura, A., & Talamo, B. R. (2006). Odorant receptors directly activate phospholipase C/inositol-1,4,5-trisphosphate coupled to calcium influx in Odora cells. *Journal of Neurochemistry*, *96*(6), 1591–1605. https://doi.org/10.1111/j.1471-4159.2006.03667.x
- Liu, X., Sambath, K., Hutnik, L., Du, J., Belfield, K. D., & Zhang, Y. (2020). Activating Acid-Sensing Ion Channels with

Photoacid Generators. *ChemPhotoChem*, *4*(12), 5337–5340. https://doi.org/10.1002/cptc.202000154

- Malchow, R. P., Tchernookova, B. K., Choi, J. V., Smith, P. J. S., Kramer, R. H., & Kreitzer, M. A. (2021). Review and Hypothesis: A Potential Common Link Between Glial Cells, Calcium Changes, Modulation of Synaptic Transmission, Spreading Depression, Migraine, and Epilepsy—H+. *Frontiers in Cellular Neuroscience*, *15*, 693095. https://doi.org/10.3389/fncel.2021.693095
- Malik, K. U. (1970). Potentiation by Anticholinesterases of the Response of Rat Mesenteric Arteries to Sympathetic Postganglionic Nerve Stimulation. *Circulation Research*, *27*(5), 647–655. https://doi.org/10.1161/01.RES.27.5.647
- Marchetta, P., Rüttiger, L., Hobbs, A. J., Singer, W., & Knipper, M. (2022). The role of cGMP signalling in auditory processing in health and disease. *British Journal of Pharmacology*, *179*(11), 2378–2393. https://doi.org/10.1111/bph.15455
- Marx, M.-C., Billups, D., & Billups, B. (2015). Maintaining the presynaptic glutamate supply for excitatory neurotransmission: Glutamate Recycling and Replenishment. *Journal of Neuroscience Research*, *93*(7), 1031–1044. https://doi.org/10.1002/jnr.23561
- Michaelson, DM & Angel, I. (1980). Determination of ΔpH in cholinergic synaptic vesicle*Life Sciences*, *27*(1), 39–44. https://doi.org/10.1016/0024-3205(80)90017-X
- Miesenbock, G & De Angelis, DA. (1998). Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins. *Nature*, *394*(6689), 192–195. https://doi.org/10.1038/28190
- Mike, A, Castro, NG, & Albuquerque, EX. (2000). Choline and acetylcholine have similar kinetic properties of activation and desensitization on the alpha7 nicotinic receptors in rat hippocampal neurons. *Brain Research*, *882*(1–2), 155–168. https://doi.org/10.1016/s0006-8993(00)02863-8
- Molinari, G. (2015). Is hydrogen ion (H+) the real second messenger in calcium signalling?*Cellular Signalling*, *27*(7), 1392–1397. https://doi.org/10.1016/j.cellsig.2015.03.023
- Molinari, G., & Nervo, E. (2021). Role of protons in calcium signaling.*Biochemical Journal*, *478*(4), 895–910. https://doi.org/10.1042/BCJ20200971
- Morelli, A. M., Ravera, S., & Panfoli, I. (2020). The aerobic mitochondrial ATP synthesis from a comprehensive point of view. *Open Biology*, *10*(10), 200224. https://doi.org/10.1098/rsob.200224
- Moreno, C. M., Dixon, R. E., Tajada, S., Yuan, C., Opitz-Araya, X., Binder, M. D., & Santana, L. F. (2016). Ca2+ entry into neurons is facilitated by cooperative gating of clustered CaV1.3 channels. *eLife*, *5*, e15744. https://doi.org/10.7554/eLife.15744
- Moro, H., Park, H.-D., & Kunito, T. (2021). Organic Phosphorus Substantially Contributes to Crop Plant Nutrition in Soils with Low Phosphorus Availability. *Agronomy*, *11*(5), 903. https://doi.org/10.3390/agronomy11050903
- Mott, D. D., Washburn, M. S., Zhang, S., & Dingledine, R. J. (2003). Subunit-Dependent Modulation of Kainate Receptors by Extracellular Protons and Polyamines. *The Journal of Neuroscience*, *23*(4), 1179–1188. https://doi.org/10.1523/JNEUROSCI.23-04-01179.2003
- Nakamura, Y., & Fukami, K. (2017). Regulation and physiological functions of mammalian phospholipase C.*Journal of Biochemistry*, *161*(4), mvw094. https://doi.org/10.1093/jb/mvw094
- Nedergaard, M. (1995). Intracellular Ca2+ transients evoked by lactic acid in cultured mammalian neurons.*American*

Journal of Physiology-Regulatory, Integrative and Comparative Physiology, *268*(2), R506–R513. https://doi.org/10.1152/ajpregu.1995.268.2.R506

- Negri, S., Scolari, F., Vismara, M., Brunetti, V., Faris, P., Terribile, G., Sancini, G., Berra-Romani, R., & Moccia, F. (2022). GABAA and GABAB Receptors Mediate GABA-Induced Intracellular Ca2+ Signals in Human Brain Microvascular Endothelial Cells. *Cells*, *11*(23), 3860. https://doi.org/10.3390/cells11233860
- Neher, E., & Sakaba, T. (2008). Multiple Roles of Calcium Ions in the Regulation of Neurotransmitter Release*Neuron*, *59*(6), 861–872. https://doi.org/10.1016/j.neuron.2008.08.019
- Newton, A. C., Bootman, M. D., & Scott, J. D. (2016). Second Messengers.*Cold Spring Harbor Perspectives in Biology*, *8*(8), a005926. https://doi.org/10.1101/cshperspect.a005926
- Nicholls, D. G., & Chalmers, S. (2004). The Integration of Mitochondrial Calcium Transport and Storage.*Journal of Bioenergetics and Biomembranes*, *36*(4), 277–281. https://doi.org/10.1023/B:JOBB.0000041753.52832.f3
- Nicholson, M. W., Sweeney, A., Pekle, E., Alam, S., Ali, A. B., Duchen, M., & Jovanovic, J. N. (2018). Diazepam-induced loss of inhibitory synapses mediated by PLCδ/ Ca2+/calcineurin signalling downstream of GABAA receptors. *Molecular Psychiatry*, *23*(9), 1851–1867. https://doi.org/10.1038/s41380-018-0100-y
- Okamura, Y., Kawanabe, A., & Kawai, T. (2018). Voltage-Sensing Phosphatases: Biophysics, Physiology, and Molecular Engineering. *Physiological Reviews*, *98*(4), 2097–2131. https://doi.org/10.1152/physrev.00056.2017
- Ooms, L. M., Horan, K. A., Rahman, P., Seaton, G., Gurung, R., Kethesparan, D. S., & Mitchell, C. A. (2009). The role of the inositol polyphosphate 5-phosphatases in cellular function and human disease. *Biochemical Journal*, *419*(1), 29–49. https://doi.org/10.1042/BJ20081673
- Orfali, R., & Albanyan, N. (2023). Ca2+-Sensitive Potassium Channels. *Molecules*, *28*(2), 885. https://doi.org/10.3390/molecules28020885
- OuYang, JB, Mellergård, P, Kristián, T, Kristiánova, V, & Siesjö, BK. (1994). Influence of acid-base changes on the intracellular calcium concentration of neurons in primary culture. *Experimental Brain Research*, *101*(2), 265–271. https://doi.org/10.1007/BF00228746
- Palmer, M. J., Hull, C., Vigh, J., & von Gersdorff, H. (2003). Synaptic Cleft Acidification and Modulation of Short-Term Depression by Exocytosed Protons in Retinal Bipolar Cells. *The Journal of Neuroscience*, *23*(36), 11332–11341. https://doi.org/10.1523/JNEUROSCI.23-36-11332.2003
- Papavassiliou, K. A., & Papavassiliou, A. G. (2021). Malignant circuits: Novel therapeutic opportunities in neuro-oncology. *Journal of Cellular and Molecular Medicine*, *25*(6), 3167–3168. https://doi.org/10.1111/jcmm.16353
- Papke, RI & Lindstrom, JM. (2020). Nicotinic acetylcholine receptors: Conventional and unconventional ligands and signaling. *Neuropharmacology*, *15*(168), 108021. https://doi.org/10.1016/j.neuropharm.2020.108021
- Pathak, D., Shields, L. Y., Mendelsohn, B. A., Haddad, D., Lin, W., Gerencser, A. A., Kim, H., Brand, M. D., Edwards, R. H., & Nakamura, K. (2015). The Role of Mitochondrially Derived ATP in Synaptic Vesicle Recycling. *Journal of Biological Chemistry*, *290*(37), 22325–22336. https://doi.org/10.1074/jbc.M115.656405
- Pozzan, T., Rizzuto, R., Volpe, P., & Meldolesi, J. (1994). Molecular and cellular physiology of intracellular calcium stores. *Physiological Reviews*, *74*(3), 595–636. https://doi.org/10.1152/physrev.1994.74.3.595
- Pulido, C & Ryan, TA. (2021). Synaptic vesicle pools are a major hidden resting metabolic burden of nerve terminals.

Science Advances, *7*(49), eabi9027. https://doi.org/10.1126/sciadv.abi9027

- Ramachandran, S., Rodgriguez, S., Potcoava, M., & Alford, S. (2022). Single Calcium Channel Nanodomains Drive Presynaptic Calcium Entry at Lamprey Reticulospinal Presynaptic Terminals. *The Journal of Neuroscience*, *42*(12), 2385–2403. https://doi.org/10.1523/JNEUROSCI.2207-21.2022
- Randall, A. S., Liu, C.-H., Chu, B., Zhang, Q., Dongre, S. A., Juusola, M., Franze, K., Wakelam, M. J. O., & Hardie, R. C. (2015). Speed and Sensitivity of Phototransduction in *Drosophila* Depend on Degree of Saturation of Membrane Phospholipids. *The Journal of Neuroscience*, *35*(6), 2731–2746. https://doi.org/10.1523/JNEUROSCI.1150-14.2015
- Rook, M. L., Musgaard, M., & MacLean, D. M. (2021). Coupling structure with function in acid-sensing ion channels: Challenges in pursuit of proton sensors. *The Journal of Physiology*, *599*(2), 417–430. https://doi.org/10.1113/JP278707
- Rossano, A. J., Chouhan, A. K., & Macleod, G. T. (2013). Genetically encoded pH-indicators reveal activity-dependent cytosolic acidification of *Drosophila* motor nerve termini *in vivo*: Genetic pH-indicators in motor nerve termini. *The Journal of Physiology*, *591*(7), 1691–1706. https://doi.org/10.1113/jphysiol.2012.248377
- Roy, G., Villar, L. M., Lazaro, I., Gonzalez, M., Bootello, A., & Gonzalez-Porque, P. (1991). Purification and properties of membrane and cytosolic phosphatidylinositol-specific phospholipases C from human spleen. *Journal of Biological Chemistry*, *266*(18), 11495–11501. https://doi.org/10.1016/S0021-9258(18)98984-2
- Ruffin, V. A., Salameh, A. I., Boron, W. F., & Parker, M. D. (2014). Intracellular pH regulation by acid-base transporters in mammalian neurons. *Frontiers in Physiology*, *5*. https://doi.org/10.3389/fphys.2014.00043
- Ruusuvuori, E., & Kaila, K. (2014). Carbonic Anhydrases and Brain pH in the Control of Neuronal Excitability. In S. C. Frost & R. McKenna (A c. Di), *Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, and Industrial Applications* (Vol. 75, pp. 271–290). Springer Netherlands. https://doi.org/10.1007/978-94-007-7359-2_14
- Rybalkin, S. D., Hinds, T. R., & Beavo, J. A. (2013). Enzyme Assays for cGMP Hydrolyzing Phosphodiesterases. In T. Krieg & R. Lukowski (A c. Di), *Guanylate Cyclase and Cyclic GMP* (Vol. 1020, pp. 51–62). Humana Press. https://doi.org/10.1007/978-1-62703-459-3_3
- Ryu, S., Liu, B., Yao, J., Fu, Q., & Qin, F. (2007). Uncoupling Proton Activation of Vanilloid Receptor TRPV1 *Journal of Neuroscience*, *27*(47), 12797–12807. https://doi.org/10.1523/JNEUROSCI.2324-07.2007
- Salucci, S., Aramini, B., Bartoletti-Stella, A., Versari, I., Martinelli, G., Blalock, W., Stella, F., & Faenza, I. (2023). Phospholipase Family Enzymes in Lung Cancer: Looking for Novel Therapeutic Approaches. *Cancers*, *15*(12), 3245. https://doi.org/10.3390/cancers15123245
- Sam, C., & Bordoni, B. (2022). *Physiology, Acetylcholine: Vol. In: StatPearls [Internet]. Treasure Island (FL)*. StatPearls Publishing. https://pubmed.ncbi.nlm.nih.gov/32491757/
- Sancho, M., & Kyle, B. D. (2021). The Large-Conductance, Calcium-Activated Potassium Channel: A Big Key Regulator of Cell Physiology. *Frontiers in Physiology*, *12*, 750615. https://doi.org/10.3389/fphys.2021.750615
- Schmid, F., Fliegert, R., Westphal, T., Bauche, A., & Guse, A. H. (2012). Nicotinic Acid Adenine Dinucleotide Phosphate (NAADP) Degradation by Alkaline Phosphatase. *Journal of Biological Chemistry*, *287*(39), 32525–32534. https://doi.org/10.1074/jbc.M112.362715
- Semtner, M., Schaefer, M., Pinkenburg, O., & Plant, T. D. (2007). Potentiation of TRPC5 by Protons *Journal of*

Biological Chemistry, *282*(46), 33868–33878. https://doi.org/10.1074/jbc.M702577200

- Shah, K. R., Guan, X., & Yan, J. (2022). Structural and Functional Coupling of Calcium-Activated BK Channels and Calcium-Permeable Channels Within Nanodomain Signaling Complexes. *Frontiers in Physiology*, *12*, 796540. https://doi.org/10.3389/fphys.2021.796540
- Sharma, A., Rahman, G., Gorelik, J., & Bhargava, A. (2023). Voltage-Gated T-Type Calcium Channel Modulation by Kinases and Phosphatases: The Old Ones, the New Ones, and the Missing Ones. *Cells*, *12*(3), 461. https://doi.org/10.3390/cells12030461
- Silbering, A. F., & Benton, R. (2010). Ionotropic and metabotropic mechanisms in chemoreception: «chance or design»? *EMBO Reports*, *11*(3), 173–179. https://doi.org/10.1038/embor.2010.8
- Silverstein, T. P. (2021). The Proton in Biochemistry: Impacts on Bioenergetics, Biophysical Chemistry, and Bioorganic Chemistry. *Frontiers in Molecular Biosciences*, *8*, 764099. https://doi.org/10.3389/fmolb.2021.764099
- Simms, B., & Zamponi, G. (2014). Neuronal voltage-gated calcium channels: Structure, function, and dysfunction. *Neuron*, *82*(1), 24–45. https://doi.org/10.1016/j.neuron.2014.03.016
- Sisignano, M., Fischer, M. J. M., & Geisslinger, G. (2021). Proton-Sensing GPCRs in Health and Disease. *Cells*, *10*(8), 2050. https://doi.org/10.3390/cells10082050
- Song, M.-K., Namgung, S. D., Choi, D., Kim, H., Seo, H., Ju, M., Lee, Y. H., Sung, T., Lee, Y.-S., Nam, K. T., & Kwon, J.-Y. (2020). Proton-enabled activation of peptide materials for biological bimodal memory. *Nature Communications*, *11*(1), 5896. https://doi.org/10.1038/s41467-020-19750-5
- Soto, E., Ortega-Ramírez, A., & Vega, R. (2018). Protons as Messengers of Intercellular Communication in the Nervous System. *Frontiers in Cellular Neuroscience*, *12*, 342. https://doi.org/10.3389/fncel.2018.00342
- Steinegger, A., Wolfbeis, O. S., & Borisov, S. M. (2020). Optical Sensing and Imaging of pH Values: Spectroscopies, Materials, and Applications. *Chemical Reviews*, *120*(22), 12357–12489. https://doi.org/10.1021/acs.chemrev.0c00451
- Storozhuk, M., Cherninskyi, A., Maximyuk, O., Isaev, D., & Krishtal, O. (2021). Acid-Sensing Ion Channels: Focus on Physiological and Some Pathological Roles in the Brain. *Current Neuropharmacology*, *19*(9), 1570–1589. https://doi.org/10.2174/1570159X19666210125151824
- Suh, Y. H., Chang, K., & Roche, K. W. (2018). Metabotropic glutamate receptor trafficking. *Molecular and Cellular Neuroscience*, *91*, 10–24. https://doi.org/10.1016/j.mcn.2018.03.014
- Sun, G. Y., Xu, J., Jensen, M. D., & Simonyi, A. (2004). Phospholipase A2 in the central nervous system. *Journal of Lipid Research*, *45*(2), 205–213. https://doi.org/10.1194/jlr.R300016-JLR200
- Sun, Y.-G., Rupprecht, V., Zhou, L., Dasgupta, R., Seibt, F., & Beierlein, M. (2016). mGluR1 and mGluR5 Synergistically Control Cholinergic Synaptic Transmission in the Thalamic Reticular Nucleus. *The Journal of Neuroscience*, *36*(30), 7886–7896. https://doi.org/10.1523/JNEUROSCI.0409-16.2016
- Svensson, E., Apergis-Schoute, J., Burnstock, G., Nusbaum, M. P., Parker, D., & Schiöth, H. B. (2019). General Principles of Neuronal Co-transmission: Insights From Multiple Model Systems. *Frontiers in Neural Circuits*, *12*, 117. https://doi.org/10.3389/fncir.2018.00117
- Swietach, P., Youm, J.-B., Saegusa, N., Leem, C.-H., Spitzer, K. W., & Vaughan-Jones, R. D. (2013). Coupled Ca^{2+}/H^{+} transport by cytoplasmic buffers regulates local Ca^{2+} and H^{+} ion signaling. *Proceedings of the National Academy of*

Sciences, *110*(22). https://doi.org/10.1073/pnas.1222433110

- Szebenyi, S. A., Ogura, T., Sathyanesan, A., AlMatrouk, A. K., Chang, J., & Lin, W. (2014). Increases in intracellular calcium via activation of potentially multiple phospholipase C isozymes in mouse olfactory neurons. *Frontiers in Cellular Neuroscience*, *8*. https://doi.org/10.3389/fncel.2014.00336
- Teng, B., Kaplan, J. P., Liang, Z., Krieger, Z., Tu, Y.-H., Burendei, B., Ward, A. B., & Liman, E. R. (2022). Structural motifs for subtype-specific pH-sensitive gating of vertebrate otopetrin proton channels. *eLife*, *11*, e77946. https://doi.org/10.7554/eLife.77946
- Thakur, D. P., Wang, Q., Jeon, J., Tian, J., & Zhu, M. X. (2020). Intracellular acidification facilitates receptor-operated TRPC4 activation through PLCδ1 in a Ca $^{2+}$ -dependent manner. *The Journal of Physiology*, *598*(13), 2651–2667. https://doi.org/10.1113/JP279658
- Tibbett, M. (2002). Considerations on the use of the p-nitrophenyl phosphomonoesterase assay in the study of the phosphorus nutrition of soil borne fungi. *Microbiological Research*, *157*(3), 221–231. https://doi.org/10.1078/0944-5013-00154
- Tivony, R., Fletcher, M., & Keyser, U. F. (2022). Quantifying proton-induced membrane polarization in single biomimetic giant vesicles. *Biophysical Journal*, *121*(12), 2223–2232. https://doi.org/10.1016/j.bpj.2022.05.041
- Tombaugh, G. C., & Somjen, G. G. (1996). Effects of extracellular pH on voltage-gated Nae, K+ and Ca2+ currents in isolated rat CAI neurons. *J Physiol*, *15*(493 (Pt3)), 719–732. https://doi.org/10.1113/jphysiol.1996.sp021417
- Tombaugh, G. C., & Somjen, G. G. (1997). Differential Sensitivity to Intracellular pH Among High- and Low-Threshold Ca $^{2+}$ Currents in Isolated Rat CA1 Neurons.*Journal of Neurophysiology*, *77*(2), 639–653. https://doi.org/10.1152/jn.1997.77.2.639
- Traynelis, S. F., & Cull-Candy, S. G. (1991). Pharmacological properties and H+ sensitivity of excitatory amino acid receptor channels in rat cerebellar granule neurones. *The Journal of Physiology*, *433*(1), 727–763. https://doi.org/10.1113/jphysiol.1991.sp018453
- Tu, Y.-H., Cooper, A. J., Teng, B., Chang, R. B., Artiga, D. J., Turner, H. N., Mulhall, E. M., Ye, W., Smith, A. D., & Liman, E. R. (2018). An evolutionarily conserved gene family encodes proton-selective ion channels. *Science*, *359*(6379), 1047–1050. https://doi.org/10.1126/science.aao3264
- Uchitel, O. D., González Inchauspe, C., & Weissmann, C. (2019). Synaptic signals mediated by protons and acid-sensing ion channels. *Synapse*, *73*(10). https://doi.org/10.1002/syn.22120
- Ueno, S., Nakaye, T., & Akaike, N. (1992). Proton-induced sodium current in freshly dissociated hypothalamic neurones of the rat. *The Journal of Physiology*, *447*(1), 309–327. https://doi.org/10.1113/jphysiol.1992.sp019004
- Verma, M., Lizama, B. N., & Chu, C. T. (2022). Excitotoxicity, calcium and mitochondria: A triad in synaptic neurodegeneration. *Translational Neurodegeneration*, *11*(1), 3. https://doi.org/10.1186/s40035-021-00278-7
- Volkov, V. I., Chernyak, A. V., Golubenko, D. V., Tverskoy, V. A., Lochin, G. A., Odjigaeva, E. S., & Yaroslavtsev, A. B. (2020). Hydration and Diffusion of H+, Li+, Na+, Cs+ Ions in Cation-Exchange Membranes Based on Polyethylene- and Sulfonated-Grafted Polystyrene Studied by NMR Technique and Ionic Conductivity Measurements. *Membranes*, *10*(10), 272. https://doi.org/10.3390/membranes10100272
- Vos, H. M. J., Zweig, R., Margenot, A. J., Koopmans, G. F., & Van Groenigen, J. W. (2023). Phosphatase activity in the

drilosphere and its link to phosphorus uptake by grass. *Geoderma*, *439*, 116690. https://doi.org/10.1016/j.geoderma.2023.116690

- Vroman, R., Klaassen, L. J., Howlett, M. H. C., Cenedese, V., Klooster, J., Sjoerdsma, T., & Kamermans, M. (2014). Extracellular ATP Hydrolysis Inhibits Synaptic Transmission by Increasing pH Buffering in the Synaptic Cleft. *PLoS Biology*, *12*(5), e1001864. https://doi.org/10.1371/journal.pbio.1001864
- Vultaggio-Poma, V., Falzoni, S., Salvi, G., Giuliani, A. L., & Di Virgilio, F. (2022). Signalling by extracellular nucleotides in health and disease. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, *1869*(5), 119237. https://doi.org/10.1016/j.bbamcr.2022.119237
- Wang, Y. Y., Chang, R. B., Allgood, S. D., Silver, W. L., & Liman, E. R. (2011). A TRPA1-dependent mechanism for the pungent sensation of weak acids. *Journal of General Physiology*, *137*(6), 493–505. https://doi.org/10.1085/jgp.201110615
- Warren, T. J., Van Hook, M. J., Supuran, C. T., & Thoreson, W. B. (2016). Sources of protons and a role for bicarbonate in inhibitory feedback from horizontal cells to cones in *Ambystoma tigrinum* retina: Protons and bicarbonate in horizontal cell feedback to cones. *The Journal of Physiology*, *594*(22), 6661–6677. https://doi.org/10.1113/JP272533
- Weernink, O., Han, L., Jakobs, KH, & Schmidt, M. (2007). Dynamic phospholipid signaling by G protein-coupled receptors. *Biochimica et Biophysica Acta*, *1768*(4), 888–900. https://doi.org/10.1016/j.bbamem.2006.09.012
- Wei, D., Mei, Y., Xia, J., & Hu, H. (2017). Orai1 and Orai3 Mediate Store-Operated Calcium Entry Contributing to Neuronal Excitability in Dorsal Root Ganglion Neurons. *Frontiers in Cellular Neuroscience*, *11*, 400. https://doi.org/10.3389/fncel.2017.00400
- Wei, W.-C., Bianchi, F., Wang, Y.-K., Tang, M.-J., Ye, H., & Glitsch, M. D. (2018). Coincidence Detection of Membrane Stretch and Extracellular pH by the Proton-Sensing Receptor OGR1 (GPR68). *Current Biology*, *28*(23), 3815-3823.e4. https://doi.org/10.1016/j.cub.2018.10.046
- Whyte-Fagundes, P., & Zoidl, G. (2018). Mechanisms of pannexin1 channel gating and regulation. *Biochimica et Biophysica Acta (BBA) - Biomembranes*, *1860*(1), 65–71. https://doi.org/10.1016/j.bbamem.2017.07.009
- Willoughby, D., & Schwiening, C. J. (2002). Electrically evoked dendritic pH transients in rat cerebellar Purkinje cells. *The Journal of Physiology*, *544*(2), 487–499. https://doi.org/10.1113/jphysiol.2002.027508
- Woll, K. A., & Van Petegem, F. (2022). Calcium-release channels: Structure and function of IP_3 receptors and ryanodine receptors. *Physiological Reviews*, *102*(1), 209–268. https://doi.org/10.1152/physrev.00033.2020
- Wollny, T., Wątek, M., Durnaś, B., Niemirowicz, K., Piktel, E., Żendzian-Piotrowska, M., Góźdź, S., & Bucki, R. (2017). Sphingosine-1-Phosphate Metabolism and Its Role in the Development of Inflammatory Bowel Disease. *International Journal of Molecular Sciences*, *18*(4), 741. https://doi.org/10.3390/ijms18040741
- Wu, L.-G., Hamid, E., Shin, W., & Chiang, H.-C. (2014). Exocytosis and Endocytosis: Modes, Functions, and Coupling Mechanisms. *Annual Review of Physiology*, *76*(1), 301–331. https://doi.org/10.1146/annurev-physiol-021113-170305
- Xue, T., Do, M. T. H., Riccio, A., Jiang, Z., Hsieh, J., Wang, H. C., Merbs, S. L., Welsbie, D. S., Yoshioka, T., Weissgerber, P., Stolz, S., Flockerzi, V., Freichel, M., Simon, M. I., Clapham, D. E., & Yau, K.-W. (2011). Melanopsin signalling in mammalian iris and retina. *Nature*, *479*(7371), 67–73. https://doi.org/10.1038/nature10567
- Yao, X., Klyukin, K., Lu, W., Onen, M., Ryu, S., Kim, D., Emond, N., Waluyo, I., Hunt, A., del Alamo, J. A., Li, J., &

Yildiz, B. (2020). Protonic solid-state electrochemical synapse for physical neural networks. *Nature Communications*, *11*(1), 3134. https://doi.org/10.1038/s41467-020-16866-6

- Ye, W., Chang, R. B., Bushman, J. D., Tu, Y.-H., Mulhall, E. M., Wilson, C. E., Cooper, A. J., Chick, W. S., Hill-Eubanks, D. C., Nelson, M. T., Kinnamon, S. C., & Liman, E. R. (2016). The K^+ channel K_{IR} 2.1 functions in tandem with proton influx to mediate sour taste transduction. *Proceedings of the National Academy of Sciences*, *113*(2). https://doi.org/10.1073/pnas.1514282112
- Young, G. S., & Kirkland, J. B. (2008). The role of dietary niacin intake and the adenosine-5′-diphosphate-ribosyl cyclase enzyme CD38 in spatial learning ability: Is cyclic adenosine diphosphate ribose the link between diet and behaviour? *Nutrition Research Reviews*, *21*(1), 42–55. https://doi.org/10.1017/S0954422408945182
- Zeng, W.-Z., Liu, D.-S., Liu, L., She, L., Wu, L.-J., & Xu, T.-L. (2015). Activation of acid-sensing ion channels by localized proton transient reveals their role in proton signaling. *Scientific Reports*, *5*(1), 14125. https://doi.org/10.1038/srep14125
- Zeng, W.-Z., & Xu, T.-L. (2012). Proton production, regulation and pathophysiological roles in the mammalian brain. *Neuroscience Bulletin*, *28*(1), 1–13. https://doi.org/10.1007/s12264-012-1068-2
- Zhang, J.-B., Chang, S., Xu, P., Miao, M., Wu, H., Zhang, Y., Zhang, T., Wang, H., Zhang, J., Xie, C., Song, N., Luo, C., Zhang, X., & Zhu, S. (2018). Structural Basis of the Proton Sensitivity of Human GluN1-GluN2A NMDA Receptors. *Cell Reports*, *25*(13), 3582-3590.e4. https://doi.org/10.1016/j.celrep.2018.11.071
- Zhang, L., Bellve, K., Fogarty, K., & Kobertz, W. R. (2016). Fluorescent Visualization of Cellular Proton Fluxes. *Cell Chemical Biology*, *23*(12), 1449–1457. https://doi.org/10.1016/j.chembiol.2016.10.013
- Zheng, S., Wang, X., Zhao, D., Liu, H., & Hu, Y. (2023). Calcium homeostasis and cancer: Insights from endoplasmic reticulum-centered organelle communications. *Trends in Cell Biology*, *33*(4), 312–323. https://doi.org/10.1016/j.tcb.2022.07.004
- Zündorf, G., & Reiser, G. (2011). Calcium Dysregulation and Homeostasis of Neural Calcium in the Molecular Mechanisms of Neurodegenerative Diseases Provide Multiple Targets for Neuroprotection. *Antioxidants & Redox Signaling*, *14*(7), 1275–1288. https://doi.org/10.1089/ars.2010.3359