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Research Article

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

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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, thus supporting the action of Na^+ and K^+ ions shown by Hodgkin and Huxley[1]. Furthermore, mechanisms are put forward concerning how many hydrolases and 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.

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1. Introduction

The importance of Na^+ and K^+ ions for nerve transmission was demonstrated by eighteen years of experimental work by Hodgkin and Huxley[1]. 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[2] (Fig.7). Subsequent studies confirmed the fundamental role of Ca^{2+} in proper transmission[3][4][5][6][7][8][9]. 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[5][10][11] and cancer[12][13][14]. 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

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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[15][16], intracellular pH transients and calcium spikes less than 2 ms. Consequently, H^+ and Ca^{2+} ions require sophisticated instruments for their study[17][18][19].

The interest in H^+ ions, identified below with the current terminology as "protons", picked up after 1980[20][21][22] and particularly with the technical progress of the last 25 years[17][18][19][23].

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[24][25] and are attracting increasing attention as neurotransmitters[26][27][28][29][30][31][32][33][34][35][36][37][38]. Protons have been shown to have an essential role at the synaptic level[39][40][41][42][35] and it has been posited that they are responsible for conduction in axons[30]. Some authors have also posited a significant role in the transmission and modulation of the signal in the nervous system generally[43][44][32][45]. 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[46][32][47][38], but they appear to be insufficient[46]. Specifically, Soto and colleagues[32] 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[48]. These problems could be overcome more simply if neurotransmitters and second messengers[49] 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 several endogenous sources of protons, which have so far been overlooked; 2) to suggest some biochemical pathways for sensory impulse initiation/transmission that can be activated by protons and Ca^{2+} ions. 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 glutamate (Glu), gamma-aminobutyric acid (GABA), adenosine 5'-triphosphate (ATP) and acetylcholine (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.

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, despite the limitations of its solvation structure, thanks to its diffusion coefficients, in bulk water[50]. 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[50]. The level of proton permeability across the phospholipid membrane is tightly controlled and depends on the lipids and proteins in the membrane[51][52][53]. 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 $\geq$ that of Na^+[54]. 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[55][56]. 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[57][51]. Also, the existence of CO_2-permeable aquaporins has been proved, but the permeation mechanism of CO_2 through aquaporins is not yet resolved[58]. Carbonic anhydrases, which have a fundamental role in proton generation from CO_2 in the whole organism, including brain[44], could be less available with regards to aquaporins[58]. Besides these routes, active transporters such as pumps and exchangers can drive protons across the plasma membrane[59][44].

The elemental charge of the proton is the same as for other individual monovalent cations, at 1.602 x 10^{-19} C. Anyway, protons can transport the charge much more quickly[60][61], via proton-hopping[62][50]. In addition to interacting with water and the three channels mentioned above, protons can modulate[63] a large variety of channels and receptors. Such as Voltage Gated Calcium Channels (VGCC/CaV) [64][65], Store Operated Calcium channels (SOC)[66], calcium-activated potassium channels (K_{Ca})[67][68], inward rectifier potassium channels (Kir)[69][70], TWIK-related acid-sensitive K^+ channel (TASK)[71], proton gated Acid Sensing Ion Channels (ASIC)[72][73] [45],multimodal Transient Receptor Potential channels (TRP)[74][75], Pannexin 1 channels (Panx1) [76], G-protein Coupled Receptors (GPCR)[77] and P2X2 purinergic receptors[78]. Furthermore, GLIC channels in prokaryotes are proton-gated. The interaction depends on the species, the extracellular or intracellular position of the protons, their concentration and the type of channel[79]. Many channels, including ASIC and TRPV1, mainly trigger activation; others, such as VGCC[80], Panx1[81], and TRPV5[82], 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[83]. 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[84]. Numerous studies on proton mobility prove that protons can move and interact in very short times with several chemical players before neutralization. Therefore, the opinion that the variations in proton concentrations are physiologically negligible because they are quickly neutralized is inexact.

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[85][86][87]. As mentioned in the introduction, a widespread lasting increase in their concentration produces the pathological condition known as acidosis[88], whilst a local and temporary increase is used currently by cells as a signal, in physiological conditions[44][32][38]. In comparison with proton and Na^+ ion, Ca^{2+} has a higher atomic mass (40 Da), carries a double positive charge and possesses much less mobility. In cells, most calcium is normally bound and the cytosolic concentration of free $Ca^{2+}$is very low. Its unique chemical characteristics have allowed calcium to become a key element in cellular signalling[89]. 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[90]. 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[86]. The acidity quickly dissolves part of the Ca^{2+} buffers and Ca^{2+} can therefore pass into the solution, producing calcium spikes[86], of intensity and duration proportional to the quantity of protons released[91][92][93][87]. 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+}[94]. Similarly, protons produce the release of other bivalent and trivalent ions, such as Zn^{2+}, Mg^{2+}, or Fe^{3+} and Mn^{3+}. 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[95] 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. The old debate between supporters of chemical versus electrical transmission[96] appears restrictive, because protons possess both capabilities.

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[97] [86]. The hydrolysis of an ester or anhydride produces an acid, in most 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)[98], IP_3 (Huang, Supplementary information, Fig.S1)[29], cAMP (Barbosa, Fig.3)[99] and cGMP (Rybalkin Fig.1)[100]. 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.

enzyme	substrate	acid product	reference
phospholipase A_2	PC	arachidonic acid	Sun[101]
phospholipase C	PIP_2	acid IP_3	Molinari, Fig.1A[97]
phospholipase D	PC	phosphatidic acid	Cazzolli[102]
ecto-ATPase	ATP	ADP + acid phosphate	Kreitzer[103]
phosphodiesterase	cAMP	acid AMP	Delhaye[104]
phosphodiesterase	cGMP	acid GMP	Delhaye[104]
cADPR cyclase	cADPR	acid ADPR	Young[105]
VSPs	phosphoinositides	acid inositol phosphate	Okamura[95]
inositol 5-phosphatase	IP_3	not yet identified*	Ooms[106]
S1P phosphatase	S1P	not yet identified*	Wollny[107]
alkaline phosphatase	NAADP	not yet identified*	Schmid[108]
acetylcholinesterase	ACh	acetic acid	Fillafer[40]

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

**See the discussion in the section below.*
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"[109].

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 doubts may arise about the acidifying power of phosphomonoesterases (phosphatases), due to the possible high pKa values of their two products: the alcohol and the inorganic acid phosphate. Phosphoric esters are stable compounds and their spontaneous hydrolysis is very slow. However, monoester monoanions with a typical second pKa of 6-7 and dianions are relatively reactive[110] (Scheme 6 and respectively Scheme 5). The reaction is a nucleophilic substitution SN_2 (P) which begins with the deprotonation of the nucleophile (water) and proceeds through a transition

state (TS) in which the phosphoryl group forms a pentacoordinate intermediate[111]. This involves a redistribution of charges and electrostatic effects[112], with consequent stabilization of the TS. Alcoholic leaving groups having pKa < 7 can promote the reaction[111][110] and release the proton after hydrolysis[111][113]. The pH rate profiles for the hydrolysis of phosphate monoesters showed maximum rates at ≈ pH 4. Protein phosphatases such as inositol phosphatase (EC 3.1.3.25) can enhance the rate of hydrolysis by a factor of ≈ 10^{21}[114]. They generally use Zn^{2+} or Mg^{2+} as cofactors[115],[116] (Fig 11, Fig 15 and Fig 16). These data taken together suggest that the physiological hydrolysis of phosphomonoesters can be acidifying, similar to triesters and diesters. Unfortunately, experimental confirmation is still lacking, as is the certain identification of the acid products of the hydrolysis of important monoesters, such as IP_3, S1P and NAADP. The acidifying power of phosphatases has so far been studied in plant roots, soil microorganisms and earthworms[117][118][119] 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 phosphodiesterases and phosphomonoesterases activity[117]. Unfortunately, the authors do not measure the contribution of phosphodiesterases and phosphomonoesterases separately.

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[120].

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[121][122][123].

> "*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*"[124].

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[125][126] for the transmission of visual stimuli[16], nociceptive stimuli[127], odor[128][129] and taste, limited to taste/flavour perceptions of sweet, bitter, umami and kokumi[130][131][132]. In these cases, the biochemical mechanism begins with the activation of a phospholipase C (PLC)[133][134] which hydrolyzes the phosphatidylinositol (4,5)-bisphosphate of the neuronal membrane. The reaction for several enzyme isoforms is pH- and Ca^{2+}-dependent[135][136][137]. This means that the reaction can be acidifying and autocatalytic[138], because the hydrolysis produces IP_3 and protons[29][97][139], which in turn produce Ca^{2+} release[140][141][142][93][138], hence promoting a rapid increase in enzymatic activity. The acidifying action has been confirmed experimentally at the presynaptic termination[143][144][19].

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[86][93]
2. Ca^{2+} release from endoplasmic reticulum stores[145]
3. Ca^{2+} influx by stimulation of the SOCs[146]

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[147][148][149][150] permeable to Ca^{2+}
- TRP[74][151][29][45] and ASIC[152] channels permeable to Ca^{2+} and Na^{+}[153].

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[154][155][156].

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[157][158] and to varying degrees to depolarization until the threshold value is reached.

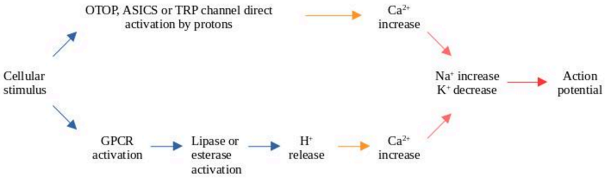
When the threshold value is exceeded Voltage Gated Sodium Channels (NaV) open, generating the action potential[159][1]. This produces the exocytosis of the vesicles and the release of the neurotransmitters into the synaptic cleft[160][15].

In the following repolarization phase the NaV channels close and the Kv[161][1][162], K_{Ca} and Hv1 proton channels[57][163] 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[5].

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[164][165]. In this case, the protons are generated by the hydrolysis of cGMP and the dissociation of acid glutamate, as described below in subsection 2.5. The role of protons in hair cell transmission is currently under debate[166].

In relation to the sensory neurons that transmit mechanical stimuli, it is believed that in mammals these neurons generally respond via mechanoelectrical channels[167]. 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[168]. In some cases, ASIC channels[169] or GPCR receptors[170] 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[171][172].

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 find a response with reference to the criticism advanced by Deng[48], according to which the Hodgkin-Huxley theory does not explain the origin of firing. The response is: 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. Scheme 1 provides a comprehensive, simplified representation of the mechanisms of proton action at the cellular level. Orange-colored arrows represent the increasing depolarization.



Scheme 1 – Mechanisms of proton actions at cellular level: in both the alternative pathways protons (H^+) are essential for the increase of Ca^{2+} concentration, depolarization and the generation of the action potential.

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, GABA and 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[173][174][175][176][177], 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.

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

Table 2. Protonated and deprotonated states of acid neurotransmitters

Therefore, it is evident that vesicle exocytosis produces inter-synaptic acidification[178][179][180][177][181][32][35] 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 the deprotonated Glu and GABA in the vesicles sufficiently rapidly to protonate and reuse them[182][183][184][185].

ATP is an important signalling molecule[186][187] as well as being a fundamental source of cellular energy, produced by mitochondria and other cellular structures[188]. 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[186]. 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[103][81] or, if in excess, cause inflammation and brain disorders[189][190][191].

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[192]. 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[193][194] and that acetylcholinesterase may be indispensable for the action of ACh[40][195]. 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[40].

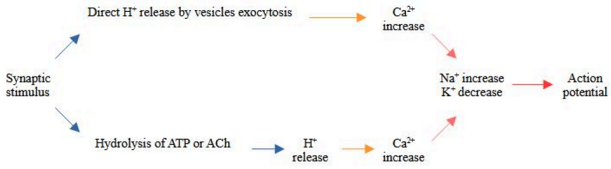
If the hypothesis that ACh can also act via its constituents were confirmed, it would be easier to clarify a number of questions that have been perplexing for some time; first of all, why there are so many different ACh receptors. In addition, the fact that the four neurotransmitters ATP, ACh, Glu and GABA can release protons explains the observation of Soto et al. regarding co-release[32], 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[196], both ionotropic such as ASICs[169][72], TRPV1[75][197][198][199], CaV3[200] and metabotropic, of the TASK type[201] and GPCRs[77]. The proton activation of the postsynaptic receptor can foster the opening of ionic channels[202][151], depolarization and the generation of a new action potential, enabling the impulse to continue[203][40][42].

Furthermore, many ligand receptors, specific for Glu, GABA and ACh, of the GPCR type, such as Group1 Glu[204][205], GABAb[7], nicotinic $\alpha 7$[63][206] and muscarinic M1, M3 and M5[207][208] receptors are activated by protons generated by PLCs. Ionotropic GABAa are also activated by the PLCs[209]. On the

contrary, most ionotropic postsynaptic receptors of glutamate are inhibited by the protons, particularly AMPARs[210], Kainate receptors[211] and NMDARs[212][213].

It is evident that protons may act at the synaptic level in various ways and via a large number of receptors. Scheme 2 provides a comprehensive, simplified representation of the possible processes of proton action at the synaptic level. Orange-colored arrows represent the increasing depolarization.



Scheme 2 – Mechanisms of proton actions at synaptic level: in both the alternative pathways protons (H^+) are essential for the increase of Ca^{2+} concentration, depolarization and the generation of the action potential.

2.6. Understanding the modes of action in depth is difficult

Fully understanding how protons can perform their neurotransmission function in each of the cases described in subsections 2.4 and 2.5 is a formidable challenge. The major difficulty is due to the high reactivity of protons, which allows very short reaction times with many different chemical species. While protons are highly mobile and reactive, they have low specificity. Therefore, it is logical to attribute to protons mainly the quantitative aspects of the mechanisms of neurotransmission, for example the changes in electrical charge and in the concentration of Ca2+. However, it cannot be ruled out that protons may also modulate some qualitative aspects through variations in the frequency, intensity and duration of the proton impulse, or through 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[214][215].

3. Conclusions

The results of the numerous experimental works cited in this review, taken together, provide an answer to the dual objective of the work and support the hypothesis that protons, with Ca^{2+} ions, may play a fundamental role in both the generation and the biochemical transmission of the nerve impulse. Protons are small, charged particles that are very mobile and can have many, different endogenous sources. At the cellular level, the transient and localized increase in protons and Ca^{2+} concentrations can activate Na^+ and K^+ channels and promote depolarization thus generating the action potential. Likewise, at the synaptic level protons and Ca^{2+} can activate post-synaptic channels and generate action potential.

These conclusions open a new perspective on neurotransmission; nevertheless, much remains to be discovered. In particular, two relevant questions require experimental answers for a better evaluation of the role of protons in neurotransmission: a) Are phosphomonoesterases able to release protons and consequently increase the Ca^{2+} concentration in physiological conditions? b) Are cholinesterases essential for the action of ACh, i.e. are the protons released by cholinesterases essential for the action of ACh? The tests to answer the two questions do not seem very difficult, especially the first one. Hopefully, somebody will perform them.

Often, experimental studies on ionic neurotransmission consider only a single step of the process of neurotransmission and a single ion. This leads to partial knowledge and the need to connect them like dominoes. For better knowledge, at least two ions should ideally be determined at the same time, in subsequent steps. The interdependence of protons and Ca^{2+} ions due to their chemical properties suggests always measuring their concentration together. The articles of Swietach et al.[87] and Liu et al.[152] can be useful examples for planning experimental works on the reciprocal roles of protons and Ca^{2+} ions in neurons. At present, several fluorescent probes are available to measure organellar pH[23] and photostimulation techniques are often used to study Ca^{2+}[7]. Mathematical models can also provide valuable help[216].

To conclude, the role of protons in neurotransmission may be more important than has so far been believed. New studies on the topic could lead to fundamental discoveries and improvements in therapeutic agents for the treatment of neurological diseases.

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References

1. a, b, c, d *Hodgkin A, Huxley AF (1952). "A quantitative description of membrane current and its application to conduction and excitation in nerve". The Journal of Physiology. 117 (4): 500–544. doi:10.1113/jphysiol.1952.sp004764.*
2. ^ *Hodgkin AL (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. doi:10.1113/jphysiol.1976.sp011620.*
3. ^ *Augustine GJ, Santamaria F, Tanaka K (2003). "Local Calcium Signaling in Neurons." Neuron. 40(2): 331–346. doi:10.1016/S0896-6273(03)00639-1.*
4. ^ *Bagur R, Hajnóczky G (2017). "Intracellular Ca2+ Sensing: Its Role in Calcium Homeostasis and Signaling." Molecular Cell. 66(6): 780–788. doi:10.1016/j.molcel.2017.05.028.*
5. a, b, c *Brini M, Calì T, Ottolini D, Carafoli E (2014). "Neuronal calcium signaling: Function and dysfunction." Cellular and Molecular Life Sciences. 71(15): 2787–2814. doi:10.1007/s00018-013-1550-7.*
6. ^ *Clapham DE. (2007). Calcium Signaling. Cell. 131 (6): 1047–1058. doi:10.1016/j.cell.2007.11.028.*
7. a, b, c *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. doi:10.3390/cells11233860.*
8. ^ *Neher E, Sakaba T. (2008). "Multiple Roles of Calcium Ions in the Regulation of Neurotransmitter Release." Neuron. 59(6): 861–872. doi:10.1016/j.neuron.2008.08.019.*
9. ^ *Pozzan T, Rizzuto R, Volpe P, Meldolesi J. (1994). "Molecular and cellular physiology of intracellular calcium stores." Physiological Reviews. 74(3): 595–636. doi:10.1152/physrev.1994.74.3.595.*
10. ^ *Manish Verma, Britney N. Lizama, Charleen T. Chu (2022). "Excitotoxicity, calcium and mitochondria: A triad in synaptic neurodegeneration." Translational Neurodegeneration. 11 (1): 3. doi:10.1186/s40035-021-00278-7.*
11. ^ *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. doi:10.1089/ars.2010.3359.*
12. ^ *Papavassiliou KA, Papavassiliou AG. (2021). "Malignant circuits: Novel therapeutic opportunities in neuro-oncology." Journal of Cellular and Molecular Medicine. 25(6): 3167–3168. doi:10.1111/jcmm.16353.*
13. ^ *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. doi:10.3390/cancers15123245.*
14. ^ *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. doi:10.1016/j.tcb.2022.07.004.*
15. a, b *Ling-Gang Wu, Edaeni Hamid, Wonchul Shin, Hsueh-Cheng Chiang (2014). "Exocytosis and Endocytosis: Modes, Functions, and Coupling Mechanisms." Annual Review of Physiology. 76 (1): 301–331. doi:10.1146/annurev-physiol-021113-170305.*
16. a, b *T. Xue, M. T. H. Do, A. Riccio, Z. Jiang, J. Hsieh, H. C. Wang, S. L. Merbs, D. S. Welsbie, T. Yoshioka, P. Weissgerber, S. Stolz, V. Flockerzi, M. Freichel, M. I. Simon, D. E. Clapham, K.-W. Yau (2011). "Melanopsin signalling in mammalian iris and retina." Nature. 479 (7371): 67–73. doi:10.1038/nature10567.*
17. a, b *Barth A, Corrie JET (2002). "Characterization of a New Caged Proton Capable of Inducing Large pH Jumps." Biophysical Journal. 83(5): 2864–2871. doi:10.1016/S0006-3495(02)75295-8.*
18. a, b *Steinegger A, Wolfbeis OS, Borisov SM. (2020). "Optical Sensing and Imaging of pH Values: Spectroscopies, Materials, and Applications." Chemical Reviews. 120(22): 12357–12489. doi:10.1021/acs.chemrev.0c00451.*
19. a, b, c *Lejie Zhang, Karl Bellve, Kevin Fogarty, William R. Kobertz (2016). "Fluorescent Visualization of Cellular Proton Fluxes." Cell Chemical Biology. 23 (12): 1449–1457. doi:10.1016/j.chembiol.2016.10.013.*
20. ^ *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. doi:10.1113/jphysiol.1991.sp018419.*

21. ^ Gruol D, Barker J, Huang L, McDonald J, Smith T Jr (1980). Hydrogen ions have multiple effects on the e xcitability of cultured mammalian neurons. 183 (1): 247–252. doi:10.1016/0006-8993(80)90138-9.
22. ^ O.A. Krishtal, V.I. Pidoplichko (1980). "A receptor fo r protons in the nerve cell membrane." Neuroscienc e. 5(12): 2325–2327. doi:10.1016/0306-4522(80)90 149-9.
23. a, b Freeman SA, Grinstein S, Orlowski J (2023). Deter minants, maintenance, and function of organellar p H. Physiol Rev. 103(1):515-606. https://doi.org/10.11 52/physrev.00009.2022
24. ^ Song MK, Namgung SD, Choi D, Kim H, Seo H, Ju M, Lee YH, Sung T, Lee YS, Nam KT, Kwon JY. (202 0). "Proton-enabled activation of peptide materials for biological bimodal memory." Nature Communic ations. 11(1): 5896. doi:10.1038/s41467-020-19750-5.
25. ^ Xiahui Yao, Konstantin Klyukin, Wenjie Lu, Murat Onen, Seungchan Ryu, Dongha Kim, Nicolas Emond, Iradwikanari Waluyo, Adrian Hunt, Jesús A. del Ala mo, Ju Li, Bilge Yildiz (2020). "Protonic solid-state e lectrochemical synapse for physical neural network s." Nature Communications. 11 (1): 3134. doi:10.103 8/s41467-020-16866-6.
26. ^ Beg AA, Ernstrom GG, Nix P, Davis MW, Jorgensen E M (2008). "Protons Act as a Transmitter for Muscle Contraction in C. elegans." Cell. 132(1): 149–160. do i:10.1016/j.cell.2007.10.058.
27. ^ Davies NW, Lux HD, Morad M. (1988). Site and mec hanism of activation of proton-induced sodium curr ent in chick dorsal root ganglion neurones. The Jour nal of Physiology. 400(1): 159–187. doi:10.1113/jphys iol.1988.sp017116.
28. ^ Diering GH, Numata M. (2014). Endosomal pH in n euronal signaling and synaptic transmission: Role of Na+/H+ exchanger NHE5. Frontiers in Physiology. 4. doi:10.3389/fphys.2013.00412.
29. a, b, c, d Huang J, Liu CH, Hughes SA, Postma M, Schw iening CJ, Hardie RC (2010). "Activation of TRP Cha nnels by Protons and Phosphoinositide Depletion in Drosophila Photoreceptors". Current Biology. 20 (3): 189–197. doi:10.1016/j.cub.2009.12.019.
30. a, b Lemont Kier (2017). "Nerve Conduction Through Dendrites via Proton Hopping." Current Computer Ai ded-Drug Design. 13(1): 57–59. doi:10.2174/1573409 912666160725113233.
31. ^ Ruffin VA, Salameh AI, Boron WF, Parker MD. (201 4). "Intracellular pH regulation by acid-base transp orters in mammalian neurons." Frontiers in Physiol ogy. 5. doi:10.3389/fphys.2014.00043.
32. a, b, c, d, e, f, g Soto E, Ortega-Ramírez A, Vega R. (201 8). "Protons as Messengers of Intercellular Commun ication in the Nervous System." Frontiers in Cellular Neuroscience. 12: 342. doi:10.3389/fncel.2018.00342.
33. ^ G C Tombaugh, G G Somjen (1996). "Effects of extra cellular pH on voltage-gated Nae, K+ and Ca2+ curr ents in isolated rat CAI neurons." J Physiol. 493 (Pt 3): 719–732. doi:10.1113/jphysiol.1996.sp021417.
34. ^ S F Traynelis, S G Cull-Candy (1991). "Pharmacolo gical properties and H+ sensitivity of excitatory ami no acid receptor channels in rat cerebellar granule n eurones." The Journal of Physiology. 433 (1): 727–76 3. doi:10.1113/jphysiol.1991.sp018453.
35. a, b, c Osvaldo D. Uchitel, Carlota González Inchausp e, Carina Weissmann (2019). "Synaptic signals medi ated by protons and acid-sensing ion channels." Syn apse. 73 (10). doi:10.1002/syn.22120.
36. ^ S Ueno, T Nakaye, N Akaike (1992). "Proton-induc ed sodium current in freshly dissociated hypothalam ic neurones of the rat." The Journal of Physiology. 44 7 (1): 309–327. doi:10.1113/jphysiol.1992.sp019004.
37. ^ Debbie Willoughby, Christof J. Schwiening (2002). "Electrically evoked dendritic pH transients in rat cer ebellar Purkinje cells." The Journal of Physiology. 54 4 (2): 487–499. doi:10.1113/jphysiol.2002.027508.
38. a, b, c Wei-Zheng Zeng, Tian-Le Xu (2012). "Proton p roduction, regulation and pathophysiological roles i n the mammalian brain." Neuroscience Bulletin. 28 (1): 1–13. doi:10.1007/s12264-012-1068-2.
39. ^ Du J, Reznikov LR, Price MP, Zha X, Lu Y, Moninger TO, Wemmie JA, Welsh MJ. (2014). Protons are a neu rotransmitter that regulates synaptic plasticity in th e lateral amygdala. Proceedings of the National Aca demy of Sciences. 111(24): 8961–8966. doi:10.1073/p nas.1407018111.
40. a, b, c, d, e Fillafer C, Koll YS, Schneider MF (2021). "Li pid Membrane State Change by Catalytic Protonatio n and the Implications for Synaptic Transmission". Membranes. 12 (1): 5. doi:10.3390/membranes12010 005.
41. ^ González-Inchauspe C, Urbano FJ, Di Guilmi MN, U chitel OD (2017). "Acid-Sensing Ion Channels Activa ted by Evoked Released Protons Modulate Synaptic T ransmission at the Mouse Calyx of Held Synapse". T he Journal of Neuroscience. 37 (10): 2589–2599. doi: 10.1523/JNEUROSCI.2566-16.2017.
42. a, b Highstein SM, Holstein GR, Mann MA, Rabbitt RD (2014). "Evidence that protons act as neurotransmitt ers at vestibular hair cell–calyx afferent synapses". P roceedings of the National Academy of Sciences. 111 (14): 5421–5426. doi:10.1073/pnas.1319561111.

43. ^ Robert Paul Malchow, Boriana K. Tchernookova, Ji-in Vivien Choi, Peter J. S. Smith, Richard H. Kramer, Matthew A. Kreitzer (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. doi:10.3389/fncel.2021.693095.
44. a, b, c, d Ruusuvuori E, Kaila K. (2014). "Carbonic Anhydrases and Brain pH in the Control of Neuronal Excitability." In: Frost SC, McKenna R, editors. Carbonic Anhydrase: Mechanism, Regulation, Links to Disease, and Industrial Applications. Vol. 75. Springer Netherlands. pp. 271–290. doi:10.1007/978-94-007-7359-2_14.
45. a, b, c Wei-Zheng Zeng, Di-Shi Liu, Lu Liu, Liang She, Long-Jun Wu, Tian-Le Xu (2015). "Activation of acid-sensing ion channels by localized proton transient reveals their role in proton signaling." Scientific Reports. 5 (1): 14125. doi:10.1038/srep14125.
46. a, b Country MW, Jonz MG. (2017). Calcium dynamics and regulation in horizontal cells of the vertebrate retina: Lessons from teleosts. Journal of Neurophysiology. 117(2): 523–536. doi:10.1152/jn.00585.2016.
47. ^ Ted J. Warren, Matthew J. Van Hook, Claudiu T. Supuran, Wallace B. Thoreson (2016). "Sources of protons and a role for bicarbonate in inhibitory feedback from horizontal cells to cones in Ambystoma tigrinum retina." The Journal of Physiology. 594 (22): 6661–6677. doi:10.1113/JP272533.
48. a, b Deng B. (2017). Alternative Models to Hodgkin-Huxley Equations. Bulletin of Mathematical Biology. 79(6): 1390–1411. doi:10.1007/s11538-017-0289-y.
49. ^ Newton AC, Bootman MD, Scott JD. (2016). "Second Messengers." Cold Spring Harbor Perspectives in Biology. 8(8): a005926. doi:10.1101/cshperspect.a005926.
50. a, b, c Silverstein TP. (2021). "The Proton in Biochemistry: Impacts on Bioenergetics, Biophysical Chemistry, and Bioorganic Chemistry." Frontiers in Molecular Biosciences. 8: 764099. doi:10.3389/fmolb.2021.764099.
51. a, b DeCoursey TE, Hosler J. (2014). Philosophy of voltage-gated proton channels. Journal of The Royal Society Interface. 11(92): 20130799. doi:10.1098/rsif.2013.0799.
52. ^ 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. doi:10.3389/fphys.2013.00382.
53. ^ Huong T. Kratochvil, Laura C. Watkins, Marco Mravic, Jessica L. Thomaston, John M. Nicoludis, Noah H. Somberg, Lijun Liu, Mei Hong, Gregory A. Voth, William F. DeGrado (2023). "Transient water wires mediate selective proton transport in designed channel proteins." Nature Chemistry. 15(7): 1012–1021. doi:10.1038/s41557-023-01210-4.
54. ^ Bozdaganyan ME, Lokhmatikov AV, Voskoboynikova N, Cherepanov DA, Steinhoff H-J, Shaitan KV, Mulkidjanian AY (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. doi:10.1016/j.bbabio.2019.03.001.
55. ^ Anderson Norris F, Powell GL (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. doi:10.1016/0005-2736(92)90269-R.
56. ^ Ran Tivony, Marcus Fletcher, Ulrich F. Keyser (2022). "Quantifying proton-induced membrane polarization in single biomimetic giant vesicles." Biophysical Journal. 121 (12): 2223–2232. doi:10.1016/j.bpj.2022.05.041.
57. a, b DeCoursey TE. (2018). Voltage and pH sensing by the voltage-gated proton channel, HV1. Journal of The Royal Society Interface. 15(141): 20180108. doi:10.1098/rsif.2018.0108.
58. a, b 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. doi:10.3389/fphys.2023.1205290.
59. ^ Doyen D, Poët M, Jarretou G, Pisani DF, 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. doi:10.3389/fmolb.2022.825028.
60. ^ Brünig FN, Rammler M, Adams EM, Havenith M, Netz RR (2022). "Spectral signatures of excess-proton waiting and transfer-path dynamics in aqueous hydrochloric acid solutions." Nature Communications. 13(1): 4210. doi:10.1038/s41467-022-31700-x.
61. ^ Vitaliy I. Volkov, Alexander V. Chernyak, Daniil V. Golubenko, Vladimir A. Tverskoy, Georgiy A. Lochin, Ervena S. Odjigaeva, Andrey B. Yaroslavtsev (2020). "Hydration and Diffusion of H+, Li+, Na+, Cs+ Ions in Cation-Exchange Membranes Based on Polyethyle

ne- and Sulfonated-Grafted Polystyrene Studied by NMR Technique and Ionic Conductivity Measureme nts." Membranes. 10 (10): 272. doi:10.3390/membra nes10100272.

62. ^ *Agmon N, Bakker HJ, Campen RK, Henchman RH, P ohl P, Roke S, Thämer M, Hassanali A (2016). "Proto ns and Hydroxide Ions in Aqueous Systems." Chemic al Reviews. 116(13): 7642–7672. doi:10.1021/acs.che mrev.5b00736.*
63. a, b *Justin R. King, Aman Ullah, Ellen Bak, M. Saleet J afri, Nadine Kabbani (2018). "Ionotropic and Metab otropic Mechanisms of Allosteric Modulation of α 7 Nicotinic Receptor Intracellular Calcium." Molecular Pharmacology. 93(6): 601–611. doi:10.1124/mol.117. 111401.*
64. ^ *Sharma A, Rahman G, Gorelik J, Bhargava A. (202 3). "Voltage-Gated T-Type Calcium Channel Modul ation by Kinases and Phosphatases: The Old Ones, th e New Ones, and the Missing Ones." Cells. 12(3): 461. doi:10.3390/cells12030461.*
65. ^ *Simms B, Zamponi G. (2014). "Neuronal voltage-g ated calcium channels: Structure, function, and dysf unction." Neuron. 82(1): 24–45. doi:10.1016/j.neuro n.2014.03.016.*
66. ^ *Robert Kraft (2015). "STIM and ORAI proteins in th e nervous system." Channels. 9(5): 245–252. doi:10.1 080/19336950.2015.1071747.*
67. ^ *Guéguinou M, Chantôme A, Fromont G, Bougnoux P, Vandier C, Potier-Cartereau M (2014). "KCa and Ca2+ channels: The complex thought". Biochimica e t Biophysica Acta (BBA) - Molecular Cell Research. 1 843 (10): 2322–2333. doi:10.1016/j.bbamcr.2014.02. 019.*
68. ^ *Sancho M, Kyle BD. (2021). "The Large-Conductan ce, Calcium-Activated Potassium Channel: A Big Key Regulator of Cell Physiology." Frontiers in Physiolog y. 12: 750615. doi:10.3389/fphys.2021.750615.*
69. ^ *Hibino H, Inanobe A, Furutani K, Murakami S, Find lay I, Kurachi Y (2010). "Inwardly Rectifying Potassi um Channels: Their Structure, Function, and Physiol ogical Roles". Physiological Reviews. 90 (1): 291–36 6. doi:10.1152/physrev.00021.2009.*
70. ^ *Wenlei Ye, Rui B. Chang, Jeremy D. Bushman, Yu-H siang Tu, Eric M. Mulhall, Courtney E. Wilson, Alexa nder J. Cooper, Wallace S. Chick, David C. Hill-Euban ks, Mark T. Nelson, Sue C. Kinnamon, Emily R. Lima n (2016). "The K+ channel KIR 2.1 functions in tande m with proton influx to mediate sour taste transduct ion." Proceedings of the National Academy of Scienc es. 113 (2). doi:10.1073/pnas.1514282112.*
71. ^ *Duprat F. (1997). TASK, a human background K+ c hannel to sense external pH variations near physiolo gical pH. The EMBO Journal. 16(17): 5464–5471. doi: 10.1093/emboj/16.17.5464.*
72. a, b *Rook ML, Musgaard M, MacLean DM. (2021). "C oupling structure with function in acid-sensing ion c hannels: Challenges in pursuit of proton sensors." Th e Journal of Physiology. 599(2): 417–430. doi:10.111 3/JP278707.*
73. ^ *Storozhuk M, Cherninskyi A, Maximyuk O, Isaev D, Krishtal O. (2021). "Acid-Sensing Ion Channels: Foc us on Physiological and Some Pathological Roles in t he Brain." Current Neuropharmacology. 19(9): 1570 –1589. doi:10.2174/1570159X19666210125151824.*
74. a, b *Cao E (2020). "Structural mechanisms of transie nt receptor potential ion channels." Journal of Gener al Physiology. 152(3): e201811998. doi:10.1085/jgp.2 01811998.*
75. a, b *Hae-Jin Kweon, Soo-Young Yu, Dong-Il Kim, By ung-Chang Suh (2015). "Differential Regulation of Proton-Sensitive Ion Channels by Phospholipids: A C omparative Study between ASICs and TRPV1." PLOS ONE. 10(3): e0122014. doi:10.1371/journal.pone.0122 014.*
76. ^ *Paige Whyte-Fagundes, Georg Zoidl (2018). "Mech anisms of pannexin1 channel gating and regulatio n." Biochimica et Biophysica Acta (BBA) - Biomemb ranes. 1860 (1): 65–71. doi:10.1016/j.bbamem.2017.0 7.009.*
77. a, b *Sisignano M, Fischer MJM, Geisslinger G. (2021). "Proton-Sensing GPCRs in Health and Disease." Cell s. 10(8): 2050. doi:10.3390/cells10082050.*
78. ^ *Burnstock G (2018). "Purine and purinergic recepto rs." Brain and Neuroscience Advances. 2: 239821281 881749. doi:10.1177/2398212818817494.*
79. ^ *de la Roche J, Eberhardt MJ, Klinger AB, Stanslowsk y N, Wegner F, Koppert W, Reeh PW, Lampert A, Fisc her MJM, Leffler A. (2013). The Molecular Basis for S pecies-specific Activation of Human TRPA1 Protein by Protons Involves Poorly Conserved Residues withi n Transmembrane Domains 5 and 6. Journal of Biol ogical Chemistry. 288(28): 20280–20292. doi:10.10 74/jbc.M113.479337.*
80. ^ *Almanza A, Mercado F, Vega R, Soto E (2008). "Ext racellular pH modulates the voltage-dependent Ca2 + current and low threshold K+ current in hair cell s." Neurochemical Research. 33(8): 1435–1441. doi:1 0.1007/s11064-007-9565-9.*
81. a, b *Rozan Vroman, Lauw J. Klaassen, Marcus H.C. Ho wlett, Valentina Cenedese, Jan Klooster, Trijntje Sjoe rdsma, Maarten Kamermans (2014). "Extracellular*

ATP Hydrolysis Inhibits Synaptic Transmission by In creasing pH Buffering in the Synaptic Cleft." PLoS Bi ology. 12 (5): e1001864. doi:10.1371/journal.pbio.100 1864.

82. ^*Fluck EC, Yazici AT, Rohacs T, Moiseenkova-Bell V Y (2022). "Structural basis of TRPV5 regulation by p hysiological and pathophysiological modulators". Ce ll Reports. 39 (4): 110737. doi:10.1016/j.celrep.2022.1 10737.*
83. ^*Catterall WA, Lenaeus MJ, Gamal El-Din TM (202 0). "Structure and Pharmacology of Voltage-Gated S odium and Calcium Channels." Annual Review of Ph armacology and Toxicology. 60(1): 133–154. doi:10.1 146/annurev-pharmtox-010818-021757.*
84. ^*Catacuzzeno L, Franciolini F (2022). "The 70-year s earch for the voltage-sensing mechanism of ion cha nnels." The Journal of Physiology. 600(14): 3227–32 47. doi:10.1113/JP282780.*
85. ^*Deplazes E, White J, Murphy C, Cranfield CG, Garcia A. (2019). Competing for the same space: Protons an d alkali ions at the interface of phospholipid bilayer s. Biophysical Reviews. 11(3): 483–490. doi:10.1007/ s12551-019-00541-2.*
86. a, b, c, d, e*Molinari G, Nervo E. (2021). "Role of proto ns in calcium signaling." Biochemical Journal. 478 (4): 895–910. doi:10.1042/BCJ20200971.*
87. a, b, c*Swietach P, Youm JB, Saegusa N, Leem CH, Spit zer KW, Vaughan-Jones RD. (2013). "Coupled Ca2+/ H+ transport by cytoplasmic buffers regulates local C a2+ and H+ ion signaling." Proceedings of the Natio nal Academy of Sciences. 110(22). doi:10.1073/pnas.1 222433110.*
88. ^*Hamroun A, Pekar JD, Lionet A, Ghulam A, Maboud ou P, Mercier A, Brousseau T, Grzych G, Glowacki F (2020). "Ionized calcium: Analytical challenges and clinical relevance". Journal of Laboratory and Precisi on Medicine. 5: 22–22. doi:10.21037/jlpm-20-60.*
89. ^*Carafoli E, Krebs J (2016). Why calcium? How calci um became the best communicator. J Biol Chem. 291 (40):20849-57. https://doi.org/10.1074/jbc.R116.735 894*
90. ^*Eisner D, Neher E, Taschenberger H, Smith G (202 3). "Physiology of intracellular calcium buffering". P hysiological Reviews. 103 (4): 2767–2845. doi:10.115 2/physrev.00042.2022.*
91. ^*Garciarena CD, Malik A, Swietach P, Moreno AP, Va ughan-Jones RD (2018). "Distinct moieties underlie biphasic H+ gating of connexin43 channels, produci ng a pH optimum for intercellular communication". The FASEB Journal. 32 (4): 1969–1981. doi:10.1096/f j.201700876R.*
92. ^*Huang S, Shen L, Roelfsema MRG, Becker D, Hedric h R (2023). "Light-gated channelrhodopsin sparks p roton-induced calcium release in guard cells". Scien ce. 382 (6676): 1314–1318. doi:10.1126/science.adj96 96.*
93. a, b, c*OuYang JB, Mellergård P, Kristián T, Kristiánov a V, Siesjö BK. (1994). "Influence of acid-base chang es on the intracellular calcium concentration of neur ons in primary culture." Experimental Brain Researc h. 101(2): 265–271. doi:10.1007/BF00228746.*
94. ^*Nicholls DG, Chalmers S. (2004). "The Integration of Mitochondrial Calcium Transport and Storage." J ournal of Bioenergetics and Biomembranes. 36(4): 2 77–281. doi:10.1023/B:JOBB.0000041753.52832.f3.*
95. a, b*Okamura Y, Kawanabe A, Kawai T. (2018). "Volt age-Sensing Phosphatases: Biophysics, Physiology, and Molecular Engineering." Physiological Reviews. 98(4): 2097–2131. doi:10.1152/physrev.00056.2017.*
96. ^*Todman D (2008). Henry Dale and the discovery of chemical synaptic transmission. Eur Neurol. 60(3):1 62-4. https://doi.org/10.1159/000145336*
97. a, b, c*Molinari G. (2015). "Is hydrogen ion (H+) the r eal second messenger in calcium signalling?" Cellula r Signalling. 27(7): 1392–1397. doi:10.1016/j.cellsig.2 015.03.023.*
98. ^*Feng PX (2017). "The Mechanism of Hydrolysis Rea ction of Adenosine Triphosphate Molecules for the G eneration of Bio-Energy and its Properties in the Liv ing Systems". International Journal of Pharmaceutic a Analytica Acta. 1 (1): 001–008.*
99. ^*Barbosa ML de C, Fumian MM, Miranda ALP de, Ba rreiro EJ, Lima LM (2011). "Therapeutic approaches for tumor necrosis factor inhibition." Brazilian Journ al of Pharmaceutical Sciences. 47(3): 427–446. doi:1 0.1590/S1984-82502011000300002.*
100. ^*Rybalkin SD, Hinds TR, Beavo JA. (2013). "Enzyme Assays for cGMP Hydrolyzing Phosphodiesterases." I n: Krieg T, Lukowski R, editors. Guanylate Cyclase a nd Cyclic GMP. Vol. 1020. Humana Press. pp. 51–62. doi:10.1007/978-1-62703-459-3_3.*
101. ^*Sun GY, Xu J, Jensen MD, Simonyi A. (2004). "Phos pholipase A2 in the central nervous system." Journal of Lipid Research. 45(2): 205–213. doi:10.1194/jlr.R3 00016-JLR200.*
102. ^*Cazzolli R, Shemon A, Fang M, Hughes W (2006). "Phospholipid signalling through phospholipase D a nd phosphatidic acid." IUBMB Life (International Un ion of Biochemistry and Molecular Biology: Life). 58 (8): 457–461. doi:10.1080/15216540600871142.*
103. a, b*Matthew A. Kreitzer, Mason Vredeveld, Kaleb Tin ner, Alyssa M. Powell, Adam W. Schantz, Rachel Lein*

inger, Rajapone Merillat, Michael W. Gongwer, Bori ana K. Tchernookova, Robert Paul Malchow (2023). "ATP-mediated Increase in H + Efflux from Retinal Müller Cells of the Axolotl." Journal of Neurophysiol ogy. jn.00321.2023. doi:10.1152/jn.00321.2023.

104. [a], [b] *Delhaye S, Bardoni B. (2021). Role of phosphodie sterases in the pathophysiology of neurodevelopmen tal disorders. Molecular Psychiatry. 26(9): 4570–45 82. doi:10.1038/s41380-020-00997-9.*
105. ^ *Genevieve S. Young, James B. Kirkland (2008). "Th e role of dietary niacin intake and the adenosine-5' -diphosphate-ribosyl cyclase enzyme CD38 in spati al learning ability: Is cyclic adenosine diphosphate ri bose the link between diet and behaviour?" Nutritio n Research Reviews. 21 (1): 42–55. doi:10.1017/S095 4422408945182.*
106. ^ *Ooms LM, Horan KA, Rahman P, Seaton G, Gurung R, Kethesparan DS, Mitchell CA. (2009). "The role of the inositol polyphosphate 5-phosphatases in cellul ar function and human disease." Biochemical Journ al. 419(1): 29–49. doi:10.1042/BJ20081673.*
107. ^ *Tomasz Wollny, Marzena Wątek, Bonita Durnaś, K atarzyna Niemirowicz, Ewelina Piktel, Małgorzata Ż endzian-Piotrowska, Stanisław Góźdź, Robert Bucki (2017). "Sphingosine-1-Phosphate Metabolism and Its Role in the Development of Inflammatory Bowel Disease." International Journal of Molecular Science s. 18 (4): 741. doi:10.3390/ijms18040741.*
108. ^ *Schmid F, Fliegert R, Westphal T, Bauche A, Guse A H. (2012). "Nicotinic Acid Adenine Dinucleotide Pho sphate (NAADP) Degradation by Alkaline Phosphata se." Journal of Biological Chemistry. 287(39): 32525 –32534. doi:10.1074/jbc.M112.362715.*
109. ^ *W. D. Kumler, John J. Eiler (1943). "The Acid Streng th of Mono and Diesters of Phosphoric Acid. The n-A lkyl Esters from Methyl to Butyl, the Esters of Biologi cal Importance, and the Natural Guanidine Phospho ric Acids." Journal of the American Chemical Society. 65(12): 2355–2361. doi:10.1021/ja01252a028.*
110. [a], [b] *Anthony J. Kirby, Faruk Nome (2015). "Fundame ntals of Phosphate Transfer." Accounts of Chemical Research. 48(7): 1806–1814. doi:10.1021/acs.account s.5b00072.*
111. [a], [b], [c] *Duarte F, Barrozo A, Åqvist J, Williams NH, Ka merlin SCL. (2016). The Competing Mechanisms of P hosphate Monoester Dianion Hydrolysis. Journal of t he American Chemical Society. 138(33): 10664–1067 3. doi:10.1021/jacs.6b06277*
112. ^ *De Vivo M, Ensing B, Dal Peraro M, Gomez GA, Chri stianson DW, Klein ML. (2007). Proton Shuttles and Phosphatase Activity in Soluble Epoxide Hydrolase. J ournal of the American Chemical Society. 129(2): 38 7–394. doi:10.1021/ja066150c.*
113. ^ *Onyido I, Obumselu OF, Egwuatu CI, Okoye NH. (20 23). "Solvent and solvation effects on reactivities an d mechanisms of phospho group transfers from phos phate and phosphinate esters to nucleophiles." Front iers in Chemistry. 11: 1176746. doi:10.3389/fchem.20 23.1176746.*
114. ^ *Chetan Lad, Nicholas H. Williams, Richard Wolfend en (2003). "The rate of hydrolysis of phosphomonoe ster dianions and the exceptional catalytic proficienc ies of protein and inositol phosphatases." Proceedin gs of the National Academy of Sciences. 100(10): 560 7–5610. doi:10.1073/pnas.0631607100.*
115. ^ *Gill R, Mohammed F, Badyal R, Coates L, Erskine P, Thompson D, Cooper J, Gore M, Wood S (2005). "Hig h-resolution structure of myo-inositol monophosph atase, the putative target of lithium therapy". Acta C rystallographica Section D Biological Crystallograph y. 61 (5): 545–555. doi:10.1107/S0907444905004038.*
116. ^ *Eiichi Kimura (2012). "Evolution of Macrocyclic Pol yamines From Molecular Science to Supramolecular Science." Bulletin of Japan Society of Coordination C hemistry. 59(0): 26–47. doi:10.4019/bjscc.59.26.*
117. [a], [b] *Moro H, Park H-D, Kunito T. (2021). "Organic Ph osphorus Substantially Contributes to Crop Plant Nu trition in Soils with Low Phosphorus Availability." A gronomy. 11(5): 903. doi:10.3390/agronomy1105090 3.*
118. ^ *Tibbett M. (2002). "Considerations on the use of th e p-nitrophenyl phosphomonoesterase assay in the study of the phosphorus nutrition of soil borne fung i." Microbiological Research. 157(3): 221–231. doi:1 0.1078/0944-5013-00154.*
119. ^ *Hannah M.J. Vos, Rachel Zweig, Andrew J. Margeno t, Gerwin F. Koopmans, Jan Willem van Groenigen (2 023). "Phosphatase activity in the drilosphere and it s link to phosphorus uptake by grass." Geoderma. 43 9: 116690. doi:10.1016/j.geoderma.2023.116690.*
120. ^ *Silbering AF, Benton R. (2010). "Ionotropic and me tabotropic mechanisms in chemoreception: 'chance or design'?" EMBO Reports. 11(3): 173–179. doi:10.10 38/embor.2010.8.*
121. ^ *Chang RB, Waters H, Liman ER (2010). "A proton c urrent drives action potentials in genetically identifi ed sour taste cells." Proceedings of the National Acad emy of Sciences. 107(51): 22320–22325. doi:10.1073/ pnas.1013664107.*
122. ^ *Teng B, Kaplan JP, Liang Z, Krieger Z, Tu YH, Buren dei B, Ward AB, Liman ER. (2022). "Structural motif*

s for subtype-specific pH-sensitive gating of vertebrate otopetrin proton channels." eLife. 11: e77946. doi:10.7554/eLife.77946.

123. ^Yu-Hsiang Tu, Alexander J. Cooper, Bochuan Teng, Rui B. Chang, Daniel J. Artiga, Heather N. Turner, Eric M. Mulhall, Wenlei Ye, Andrew D. Smith, Emily R. Liman (2018). "An evolutionarily conserved gene family encodes proton-selective ion channels." Science. 359 (6379): 1047–1050. doi:10.1126/science.aao3264.

124. ^Emily R Liman, Sue C Kinnamon (2021). "Sour taste: Receptors, cells and circuits." Current Opinion in Physiology. 20: 8–15. doi:10.1016/j.cophys.2020.12.006.

125. ^Pedro H. Imenez Silva, Carsten A. Wagner (2022). "Physiological relevance of proton-activated GPCRs." Pflügers Archiv - European Journal of Physiology. 474(5): 487–504. doi:10.1007/s00424-022-02671-1.

126. ^Federica Liccardo, Alberto Luini, Rosaria Di Martino (2022). "Endomembrane-Based Signaling by GPCRs and G-Proteins." Cells. 11(3): 528. doi:10.3390/cells11030528.

127. ^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. doi:10.1016/j.neuron.2015.11.001.

128. ^Guang Liu, Robert M. Badeau, Akihiko Tanimura, Barbara R. Talamo (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. doi:10.1111/j.1471-4159.2006.03667.x.

129. ^Szebenyi SA, Ogura T, Sathyanesan A, AlMatrouk AK, Chang J, Lin W. (2014). "Increases in intracellular calcium via activation of potentially multiple phosphoholipase C isozymes in mouse olfactory neurons." Frontiers in Cellular Neuroscience. 8. doi:10.3389/fncel.2014.00336.

130. ^Ahmad R, Dalziel JE (2020). "G Protein-Coupled Receptors in Taste Physiology and Pharmacology." Frontiers in Pharmacology. 11: 587664. doi:10.3389/fphar.2020.587664.

131. ^Deshpande DA, Wang WCH, McIlmoyle EL, Robinett KS, Schillinger RM, An SS, Sham JSK, Liggett SB. (2010). Bitter taste receptors on airway smooth muscle bronchodilate by localized calcium signaling and reverse obstruction. Nature Medicine. 16(11): 1299–1304. doi:10.1038/nm.2237.

132. ^Allen Lee, Chung Owyang (2017). "Sugars, Sweet Taste Receptors, and Brain Responses." Nutrients. 9 (7): 653. doi:10.3390/nu9070653.

133. ^Balla T (2010). "Putting G protein–coupled receptor-mediated activation of phospholipase C in the limelight." Journal of General Physiology. 135(2): 77–80. doi:10.1085/jgp.200910396.

134. ^Paschal A. Oude Weernink, Li Han, Karl H. Jakobs, Martina Schmidt (2007). "Dynamic phospholipid signaling by G protein-coupled receptors." Biochimica et Biophysica Acta. 1768 (4): 888–900. doi:10.1016/j.bbamem.2006.09.012.

135. ^Banno Y, Nozawa Y (1987). "Characterization of partially purified phospholipase C from human platelet membranes." Biochemical Journal. 248(1): 95–101. doi:10.1042/bj2480095.

136. ^Nakamura Y, Fukami K. (2017). "Regulation and physiological functions of mammalian phospholipase C." Journal of Biochemistry. 161(4): mvw094. doi:10.1093/jb/mvw094.

137. ^Roy G, Villar LM, 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. doi:10.1016/S0021-9258(18)98984-2.

138. a, bThakur DP, Wang Q, Jeon J, Tian JB, Zhu MX. (2020). "Intracellular acidification facilitates receptor-operated TRPC4 activation through PLCδ1 in a Ca2+-dependent manner." The Journal of Physiology. 598 (13): 2651–2667. doi:10.1113/JP279658.

139. ^Randall AS, Liu C-H, Chu B, Zhang Q, Dongre SA, Juusola M, Franze K, Wakelam MJO, Hardie RC. (2015). "Speed and Sensitivity of Phototransduction in Drosophila Depend on Degree of Saturation of Membrane Phospholipids." The Journal of Neuroscience. 35 (6): 2731–2746. doi:10.1523/JNEUROSCI.1150-14.2015.

140. ^Chen WH, Chen CR, Yang KT, Chang WL, Su MJ, Wu CC, Wu ML. (2001). Arachidonic acid-induced H+ and Ca2+ increases in both the cytoplasm and nucleoplasm of rat cerebellar granule cells. The Journal of Physiology. 537(2): 497–510. doi:10.1111/j.1469-7793.2001.00497.x.

141. ^David Križaj, Aaron J. Mercer, Wallace B. Thoreson, Peter Barabas (2011). "Intracellular pH modulates inner segment calcium homeostasis in vertebrate photoreceptors." American Journal of Physiology-Cell Physiology. 300(1): C187–C197. doi:10.1152/ajpcell.00264.2010.

142. ^Nedergaard M. (1995). "Intracellular Ca2+ transients evoked by lactic acid in cultured mammalian neurons." American Journal of Physiology-Regulatory, I

ntegrative and Comparative Physiology. 268(2): R506–R513. doi:10.1152/ajpregu.1995.268.2.R506.

143. ^*Caldwell L, Harries P, Sydlik S, Schwiening CJ (2013). "Presynaptic pH and vesicle fusion in Drosophila larvae neurones." Synapse. 67(11): 729–740. doi:10.1002/syn.21678.*
144. ^*Rossano AJ, Chouhan AK, Macleod GT. (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. doi:10.1113/jphysiol.2012.248377.*
145. ^*Kellie A. Woll, Filip Van Petegem (2022). "Calcium-release channels: Structure and function of IP3 receptors and ryanodine receptors." Physiological Reviews. 102 (1): 209–268. doi:10.1152/physrev.00033.2020.*
146. ^*Dongyu Wei, Yixiao Mei, Jingsheng Xia, Huijuan Hu (2017). "Orai1 and Orai3 Mediate Store-Operated Calcium Entry Contributing to Neuronal Excitability in Dorsal Root Ganglion Neurons." Frontiers in Cellular Neuroscience. 11: 400. doi:10.3389/fncel.2017.00400.*
147. ^*Dolphin AC. (2020). Functions of Presynaptic Voltage-gated Calcium Channels. Function. 2(1): zqaa027. doi:10.1093/function/zqaa027.*
148. ^*Harding EK, Zamponi GW (2022). "Central and peripheral contributions of T-type calcium channels in pain". Molecular Brain. 15 (1): 39. doi:10.1186/s13041-022-00923-w.*
149. ^*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. doi:10.1523/JNEUROSCI.2207-21.2022.*
150. ^*Geoffrey C. Tombaugh, George G. Somjen (1997). "Differential Sensitivity to Intracellular pH Among High- and Low-Threshold Ca2+ Currents in Isolated Rat CA1 Neurons." Journal of Neurophysiology. 77 (2): 639–653. doi:10.1152/jn.1997.77.2.639.*
151. ^a, ^b*Henrich M, Buckler KJ (2009). "Acid-evoked Ca2+ signalling in rat sensory neurones: Effects of anoxia and aglycaemia". Pflügers Archiv - European Journal of Physiology. 459 (1): 159–181. doi:10.1007/s00424-009-0715-6.*
152. ^a, ^b*Xinglei Liu, Karthik Sambath, Lauren Hutnik, Jianyang Du, Kevin D. Belfield, Yuanwei Zhang (2020). "Activating Acid-Sensing Ion Channels with Photoacid Generators." ChemPhotoChem. 4(12): 5337–5340. doi:10.1002/cptc.202000154.*
153. ^*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. doi:10.3892/etm.2021.10897.*
154. ^*Hou S, Xu R, Heinemann SH, 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. doi:10.1038/nsmb.1398.*
155. ^*Orfali R, Albanyan N. (2023). "Ca2+-Sensitive Potassium Channels." Molecules. 28(2): 885. doi:10.3390/molecules28020885.*
156. ^*Shah KR, 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. doi:10.3389/fphys.2021.796540.*
157. ^*Dixon RE, Navedo MF, Binder MD, Santana LF. (2022). Mechanisms and physiological implications of cooperative gating of clustered ion channels. Physiological Reviews. 102(3): 1159–1210. doi:10.1152/physrev.00022.2021.*
158. ^*Moreno CM, Dixon RE, Tajada S, Yuan C, Opitz-Araya X, Binder MD, Santana LF. (2016). "Ca2+ entry into neurons is facilitated by cooperative gating of clustered CaV1.3 channels." eLife. 5: e15744. doi:10.7554/eLife.15744.*
159. ^*Catterall WA, Goldin AL, Waxman SG (2005). "International Union of Pharmacology. XLVII. Nomenclature and Structure-Function Relationships of Voltage-Gated Sodium Channels." Pharmacological Reviews. 57(4): 397–409. doi:10.1124/pr.57.4.4.*
160. ^*Ge L, Shin W, Arpino G, Wei L, Chan CY, Bleck CKE, Zhao W, Wu LG (2022). "Sequential compound fusion and kiss-and-run mediate exo- and endocytosis in excitable cells". Science Advances. 8 (24): eabm6049. doi:10.1126/sciadv.abm6049.*
161. ^*Grider MH, 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/.*
162. ^*Alisher M. Kariev, Michael E. Green (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. doi:10.3390/membranes12070718.*
163. ^*Han S, Peng S, Vance J, Tran K, Do N, Bui N, Gui Z, Wang S (2022). "Structural dynamics determine volt*

age and pH gating in human voltage-gated proton c hannel". eLife. 11: e73093. doi:10.7554/eLife.73093.

164. ^*Chen CK, Woodruff ML, Fain GL. (2015). Rhodopsin kinase and recoverin modulate phosphodiesterase d uring mouse photoreceptor light adaptation. Journal of General Physiology. 145(3): 213–224. doi:10.1085/ jgp.201411273.*
165. ^*Philine Marchetta, Lukas Rüttiger, Adrian J. Hobbs, Wibke Singer, Marlies Knipper (2022). "The role of c GMP signalling in auditory processing in health and disease." British Journal of Pharmacology. 179(11): 2 378–2393. doi:10.1111/bph.15455.*
166. ^*Contini D, Holstein GR, Art JJ. (2022). Simultaneous Dual Recordings From Vestibular Hair Cells and Thei r Calyx Afferents Demonstrate Multiple Modes of Tra nsmission at These Specialized Endings. Frontiers in Neurology. 13: 891536. doi:10.3389/fneur.2022.8915 36.*
167. ^*Douguet D, Honoré E. (2019). Mammalian Mechan oelectrical Transduction: Structure and Function of F orce-Gated Ion Channels. Cell. 179(2): 340–354. doi: 10.1016/j.cell.2019.08.049.*
168. ^*Bavi N, Nikolaev YA, Bavi O, Ridone P, Martinac A D, Nakayama Y, Cox CD, Martinac B (2017). "Princip les of Mechanosensing at the Membrane Interface." In R. M. Epand & J.-M. Ruysschaert (A c. Di), The Bio physics of Cell Membranes (Vol. 19, pp. 85–119). Spri nger Singapore. doi:10.1007/978-981-10-6244-5_ 4.*
169. a, b*Cheng YR, Jiang BY, Chen CC. (2018). Acid-sensin g ion channels: Dual function proteins for chemo-se nsing and mechano-sensing. Journal of Biomedical Science. 25(1): 46. doi:10.1186/s12929-018-0448-y.*
170. ^*Hsi-Hsien Lin, Kwai-Fong Ng, Tse-Ching Chen, W en-Yi Tseng (2022). "Ligands and Beyond: Mechan osensitive Adhesion GPCRs." Pharmaceuticals. 15(2): 219. doi:10.3390/ph15020219.*
171. ^*Adam J. Iliff, X.Z. Shawn Xu (2018). "A Mechanosen sitive GPCR that Detects the Bloody Force." Cell. 173 (3): 542–544. doi:10.1016/j.cell.2018.04.001.*
172. ^*Wei-Chun Wei, Fabio Bianchi, Yang-Kao Wang, Mi ng-Jer Tang, Hua Ye, Maike D. Glitsch (2018). "Coin cidence Detection of Membrane Stretch and Extracel lular pH by the Proton-Sensing Receptor OGR1 (GPR 68)." Current Biology. 28 (23): 3815-3823.e4. doi:10. 1016/j.cub.2018.10.046.*
173. ^*Anderson RG, Orci L (1988). "A view of acidic intrac ellular compartments." Journal of Cell Biology. 106 (3): 539–543. doi:10.1083/jcb.106.3.539.*
174. ^*Egashira Y, Takase M, Watanabe S, Ishida J, Fukam izu A, Kaneko R, Yanagawa Y, Takamori S. (2016). U nique pH dynamics in GABAergic synaptic vesicles ill uminates the mechanism and kinetics of GABA loadi ng. Proceedings of the National Academy of Science s. 113(38): 10702–10707. doi:10.1073/pnas.16045271 13.*
175. ^*Fuldner HH, Stadler H (1982). "31P-NMR Analysis of Synaptic Vesicles". European Journal of Biochemis try. 121: 519–524.*
176. ^*Michaelson DM, Angel I. (1980). "Determination of ⊿pH in cholinergic synaptic vesicle." Life Sciences. 27 (1): 39–44. doi:10.1016/0024-3205(80)90017-X.*
177. a, b*Miesenböck G, De Angelis DA, Rothman JE. (199 8). "Visualizing secretion and synaptic transmission with pH-sensitive green fluorescent proteins." Natur e. 394(6689): 192–195. doi:10.1038/28190.*
178. ^*Ahdut-Hacohen R, Duridanova D, Meiri H, Rahami moff R (2004). "Hydrogen ions control synaptic vesi cle ion channel activity in Torpedo electromotor neu rones: H+ dependence of synaptic vesicle ion channe ls." The Journal of Physiology. 556(2): 347–352. doi: 10.1113/jphysiol.2003.058818.*
179. ^*DeVries SH. (2001). Exocytosed Protons Feedback t o Suppress the Ca2+ Current in Mammalian Cone Ph otoreceptors. Neuron. 32(6): 1107–1117. doi:10.1016/ S0896-6273(01)00535-9.*
180. ^*Bettina Kolen, Bart Borghans, Daniel Kortzak, Victo r Lugo, Cora Hannack, Raul E. Guzman, Ghanim Ulla h, Christoph Fahlke (2023). "Vesicular glutamate tra nsporters are H+-anion exchangers that operate at variable stoichiometry." Nature Communications. 14 (1): 2723. doi:10.1038/s41467-023-38340-9.*
181. ^*Palmer MJ, Hull C, Vigh J, von Gersdorff H. (2003). "Synaptic Cleft Acidification and Modulation of Shor t-Term Depression by Exocytosed Protons in Retinal Bipolar Cells." The Journal of Neuroscience. 23(36): 1 1332–11341. doi:10.1523/JNEUROSCI.23-36-11332.2 003.*
182. ^*Eriksen J, Chang R, McGregor M, Silm K, Suzuki T, Edwards RH (2016). "Protons Regulate Vesicular Glu tamate Transporters through an Allosteric Mechanis m". Neuron. 90 (4): 768–780. doi:10.1016/j.neuron. 2016.03.026.*
183. ^*Mari-Carmen Marx, Daniela Billups, Brian Billups (2015). "Maintaining the presynaptic glutamate sup ply for excitatory neurotransmission: Glutamate Rec ycling and Replenishment." Journal of Neuroscience Research. 93(7): 1031–1044. doi:10.1002/jnr.23561.*
184. ^*Pathak D, Shields LY, Mendelsohn BA, Haddad D, L in W, Gerencser AA, Kim H, Brand MD, Edwards RH, Nakamura K. (2015). "The Role of Mitochondrially D erived ATP in Synaptic Vesicle Recycling." Journal of*

Biological Chemistry. 290(37): 22325–22336. doi:10.1074/jbc.M115.656405.

185. ^*Pulido C, Ryan TA. (2021). "Synaptic vesicle pools are a major hidden resting metabolic burden of nerve terminals." Science Advances. 7(49): eabi9027. doi:10.1126/sciadv.abi9027.*
186. a, b*Burnstock G (2020). "Introduction to Purinergic Signaling." In P. Pelegrín (A c. Di), Purinergic Signaling (Vol. 2041, pp. 1–15). Springer New York. doi:10.1007/978-1-4939-9717-6_1.*
187. ^*Dunn J, Grider MH. (2023). Physiology, Adenosine Triphosphate. In StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK553175/*
188. ^*Morelli AM, Ravera S, Panfoli I. (2020). "The aerobic mitochondrial ATP synthesis from a comprehensive point of view." Open Biology. 10(10): 200224. doi:10.1098/rsob.200224.*
189. ^*Di Virgilio F, Vultaggio-Poma V, Falzoni S, Giuliani AL. (2023). Extracellular ATP: A powerful inflammatory mediator in the central nervous system. Neuropharmacology. 224: 109333. doi:10.1016/j.neuropharm.2022.109333.*
190. ^*Dias L, Pochmann D, Lemos C, Silva HB, Real JI, Gonçalves FQ, Rial D, Gonçalves N, Simões AP, Ferreira SG, Agostinho P, Cunha RA, Tomé AR. (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. doi:10.1021/acschemneuro.2c00810.*
191. ^*Valentina Vultaggio-Poma, Simonetta Falzoni, Giada Salvi, Anna Lisa Giuliani, Francesco Di Virgilio (2022). "Signalling by extracellular nucleotides in health and disease." Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 1869 (5): 119237. doi:10.1016/j.bbamcr.2022.119237.*
192. ^*KAFAIT U. MALIK (1970). "Potentiation by Antichol inesterases of the Response of Rat Mesenteric Arteries to Sympathetic Postganglionic Nerve Stimulation." Circulation Research. 27(5): 647–655. doi:10.1161/01.RES.27.5.647.*
193. ^*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. doi:10.1016/s0006-8993(00)02863-8.*
194. ^*Yuanyuan Y. Wang, Rui B. Chang, Sallie D. Allgood, Wayne L. Silver, Emily R. Liman (2011). "A TRPA1-dependent mechanism for the pungent sensation of weak acids." Journal of General Physiology. 137 (6): 493–505. doi:10.1085/jgp.201110615.*
195. ^*Fillafer C, Schneider MF (2016). "On the excitation of action potentials by protons and its potential implications for cholinergic transmission". Protoplasma. 253 (2): 357–365. doi:10.1007/s00709-015-0815-4.*
196. ^*Holzer P (2011). "Acid sensing by visceral afferent neurones: Acid sensing by visceral afferent neurones". Acta Physiologica. 201 (1): 63–75. doi:10.1111/j.1748-1716.2010.02143.x.*
197. ^*A. Leffler, B. Mönter, M. Koltzenburg (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. doi:10.1016/j.neuroscience.2005.12.020.*
198. ^*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. doi:10.1523/JNEUROSCI.2324-07.2007.*
199. ^*Semtner M, Schaefer M, Pinkenburg O, Plant TD. (2007). "Potentiation of TRPC5 by Protons." Journal of Biological Chemistry. 282(46): 33868–33878. doi:10.1074/jbc.M702577200.*
200. ^*Anna M. Lipkin, Margaret M. Cunniff, Perry W. E. Spratt, Stefan M. Lemke, Kevin J. Bender (2021). "Functional Microstructure of Ca V -Mediated Calcium Signaling in the Axon Initial Segment." The Journal of Neuroscience. 41(17): 3764–3776. doi:10.1523/JNEUROSCI.2843-20.2021.*
201. ^*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. doi:10.3390/molecules27238296.*
202. ^*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. doi:10.1016/j.mcn.2014.04.007.*
203. ^*Burke KJ, Bender KJ (2019). "Modulation of Ion Channels in the Axon: Mechanisms and Function." Frontiers in Cellular Neuroscience. 13: 221. doi:10.3389/fncel.2019.00221.*
204. ^*Suh YH, Chang K, Roche KW. (2018). "Metabotropic glutamate receptor trafficking." Molecular and Cellular Neuroscience. 91: 10–24. doi:10.1016/j.mcn.2018.03.014.*
205. ^*Sun YG, 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 Neuro*

science. 36(30): 7886–7896. doi:10.1523/JNEUROSCI.0409-16.2016.

206. ^*Papke RI, Lindstrom JM. (2020). "Nicotinic acetylcholine receptors: Conventional and unconventional ligands and signaling." Neuropharmacology. 15(168): 108021. doi:10.1016/j.neuropharm.2020.108021.*
207. ^*Brown DA (2019). "Acetylcholine and cholinergic receptors." Brain and Neuroscience Advances. 3: 2398212818820506. doi:10.1177/2398212818820506.*
208. ^*Sam C, Bordoni B. (2022). Physiology, Acetylcholine: Vol. In: StatPearls [Internet]. Treasure Island (FL). StatPearls Publishing. https://pubmed.ncbi.nlm.nih.gov/32491757/.*
209. ^*Nicholson MW, Sweeney A, Pekle E, Alam S, Ali AB, Duchen M, Jovanovic JN. (2018). "Diazepam-induced loss of inhibitory synapses mediated by PLCδ/ Ca2+/calcineurin signalling downstream of GABAA receptors." Molecular Psychiatry. 23(9): 1851–1867. doi:10.1038/s41380-018-0100-y.*
210. ^*Eva C. Ihle, Doris K. Patneau (2000). "Modulation of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid Receptor Desensitization by Extracellular Protons." Molecular Pharmacology. 58(6): 1204–1212. doi:10.1124/mol.58.6.1204.*
211. ^*Mott DD, Washburn MS, Zhang S, Dingledine RJ. (2003). "Subunit-Dependent Modulation of Kainate Receptors by Extracellular Protons and Polyamines." The Journal of Neuroscience. 23(4): 1179–1188. doi:10.1523/JNEUROSCI.23-04-01179.2003.*
212. ^*Dravid SM, Erreger K, Yuan H, Nicholson K, Le P, Lyuboslavsky P, Almonte A, Murray E, Mosley C, Barber J, French A, Balster R, Murray TF, Traynelis SF. (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. doi:10.1113/jphysiol.2006.124958.*
213. ^*Jin-Bao Zhang, Shenghai Chang, Pan Xu, Miao Miao, Hangjun Wu, Youyi Zhang, Tongtong Zhang, Han Wang, Jilin Zhang, Chun Xie, Nan Song, Cheng Luo, Xing Zhang, Shujia Zhu (2018). "Structural Basis of the Proton Sensitivity of Human GluN1-GluN2A NMDA Receptors." Cell Reports. 25 (13): 3582-3590.e4. doi:10.1016/j.celrep.2018.11.071.*
214. ^*Patrick J. Hunt, Mikhail Kochukov, Brandon T. Pekarek, Benjamin D.W. Belfort, Juan M. Romero, Jessica L. Swanson, Benjamin R. Arenkiel (2022). "Co-transmitting neurons in the lateral septal nucleus exhibit features of neurotransmitter switching." IBRO Neuroscience Reports. 12: 390–398. doi:10.1016/j.ibneur.2022.05.003.*
215. ^*Svensson E, Apergis-Schoute J, Burnstock G, Nusbaum MP, Parker D, Schiöth HB. (2019). "General Principles of Neuronal Co-transmission: Insights From Multiple Model Systems." Frontiers in Neural Circuits. 12: 117. doi:10.3389/fncir.2018.00117.*
216. ^*King J, Eroumé KS, Truckenmüller R, Giselbrecht S, Cowan AE, Loew L, Carlier A (2021). Ten steps to investigate a cellular system with mathematical modeling. PLoS Comput Biol. 17(5):e1008921. https://doi.org/10.1371/journal.pcbi.1008921*

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