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Physiological carbon dioxide, bicarbonate, and pH sensing

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Abstract

In biological systems, carbon dioxide exists in equilibrium with bicarbonate and protons. The individual components of this equilibrium (i.e., CO_2, HCO_3^-, and H^+), which must be sensed to be able to maintain cellular and organismal pH, also function as signals to modulate multiple physiological functions. Yet, the molecular sensors for CO_2/HCO_3^-/pH remained unknown until recently. Here, we review recent progress in delineating molecular and cellular mechanisms for sensing CO_2, HCO_3^-, and pH.

Keywords

Adenylyl cyclase; Bicarbonate; Carbon dioxide; Carbonic anhydrase; Channels; pH; Protons; Sensory transduction

Carbon dioxide (CO_2), bicarbonate ions (HCO_3^-), and pH/ protons (H^+) are inextricably linked in biological systems. Due to ubiquitous carbonic anhydrases, CO_2 is in nearly instantaneous equilibrium with its hydrated form H_2CO_3, which in turn rapidly dissociates into H^+ and HCO_3^-. Consequently, changes to any one of these molecules are reflected by variations in the other two, and all three (CO_2, HCO_3^-, and pH) play central roles in biology. Cellular enzymes and chemical reactions are sensitive to pH, and cells actively transport H^+ and HCO_3^- across their cell membrane to maintain intracellular pH (pH_i) (reviewed in [1,2]). Plus, CO_2/HCO_3^- buffers intracellular and extracellular physiological fluids and is the essential substrates or end products of biological calcification, photosynthesis, and respiration. Thus, cellular homeostasis and organismal homeostasis depend on sensing and tightly regulating levels of CO_2, HCO_3^-, and H^+.

While all cells must possess mechanisms to sense, and respond to, the levels of CO_2, HCO_3^-, and H^+, multicellular organisms have specialized cells which measure intracellular HCO_3^- ($[HCO_3^-]_i$) and pH_i as surrogates for the levels of CO_2, pH (pH_e), and $[HCO_3^-]$ ($[HCO_3^-]_e$) in their immediate environment. In these sensory cells, variations in pH_i or $[HCO_3^-]_i$ elicit such diverse responses as secretion/absorption of H^+, HCO_3^-, and other ions, as adjustments in lung ventilation rate, as changes in metabolism, and as regulation of gene expression.

Implicit in the definition of a biological sensor, in addition to “sensing” at least one of the relevant variables, a CO_2, HCO_3^-, or pH sensor must trigger appropriate downstream responses. This signaling function can be achieved by altering membrane potential, by producing a second messenger molecule, or by modulating other proteins and enzymes via

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allosteric conformational changes or posttranslational modifications such as phosphorylation/dephosphorylation. In this review, we describe recent advances about how molecular pH, HCO_3^-, and/or CO_2 sensors function.

pH sensors

Extracellular pH (pH_e) sensors

Organisms sense pH_e to maintain and restore systemic acid/ base balance, to adjust cellular metabolism to environmental conditions, and to detect and transduce sensory stimuli.

At least three G protein-coupled receptors (GPCRs) are activated by physiological increases in extracellular $[H^+]$ (decreases in pH_e): OGR1, GPR4, and TDAG8. In general, these pH_e-sensing GPCRs are inactive at $pH_e>7.5$ and are fully activated at $pH_e<6.8$. The mechanism of activation by H^+ probably involves the protonation of histidines exposed to the extracellular medium [3,4], inducing conformational changes that promote activation of the particular downstream heterotrimeric G proteins. The end results are increased production of the intracellular second messengers cAMP (via G_s stimulation of transmembrane adenylyl cyclase activity) or IP_3 and diacylglycerol (via G_q family stimulation of phospholipase C activity).

Ovarian cancer G protein-coupled receptor 1(OGR1, GPR68) stimulation of IP_3 production has been reported in osteoblasts [3] and osteoclasts [5], where it is thought to play roles in differentiation, metabolism, and systemic pH homeostasis [3,5–8], as well as in aortic smooth muscle cells [6], where it is thought to mediate vasorelaxation via production of prostaglandin I_2 [7]. OGR1-dependent IP_3 formation is inactive at pH_e 7.8 and maximally active at pH_e 6.8 [3]. Activation of OGR1 also leads to the formation of cAMP in aortic smooth muscle cells [6]; however, this cAMP increase may be secondary to the IP_3-elicited increase in intracellular calcium, rather than through direct G_s stimulation of a transmembrane adenylyl cyclase. Additionally, OGR1 is detectable in virtually every tissue tested and has been specifically identified in lung, kidney, and nervous system [3,6,8], suggesting there are additional roles of pH_e sensing.

GPR4 (aka GPRC6.1 [9]) is predominantly found in lung, although it is also present in kidney, heart, liver, skeletal muscle, ovary, and placenta [8–10]. In an overexpression system, GPR4 was linked to the activation of G_s and production of cAMP with pH responsiveness similar to OGR1 [3]; however, its signaling in more physiologically relevant contexts (i.e., aortic smooth muscle cells) remains unclear [7]. GPR4 is important for regulating angiogenesis [10], and it may also be important for the inflammatory response in endothelial cells [8].

The third H^+-sensing GPCR is T cell death-associated gene (8TDAG8, GPR65), which seems to be exclusively present in immune cells and lymphoid tissues [6,11]. Activation of TDAG8 by acidic pH_e leads the production of cAMP in a variety of transfected cultured cell lines [4, 12,13] as well as in thymocytes and splenocytes [13]. TDAG8 overexpression in thymocytes promoted glucocorticoid-induced apoptosis [14]. TDAG8 genetic knockdown did not affect immune development or glucocorticoid-induced apoptosis [15]; however, the lack of phenotype may be due to compensation by other pH-sensing GPCRs or redundant pH-sensing mechanisms.

In addition to the H^+-sensing GPCRs, pH_e is also sensed via H^+-sensitive ion channels, for example, some inwardly rectifying K^+ channels are regulated by intra- and/or extracellular pH. In general, acidic pH inhibits channel current while alkaline pH enhances it (reviewed in [16]). The better-characterized pH-sensitive K^+ channels are the rat outer medullary K^+ channel (ROMK) [17] and the TWIK-related acid-sensitive K^+ channel (TASK) [18]. ROMK, TASK, and related inwardly rectifying K^+ channels have been proposed to function as pH sensors

throughout the body (especially in the kidney and in the brain stem) by modulating cell membrane potential (recently reviewed in [16,19,20]). Other H^+-sensitive ion channels are predominantly found in sensory neurons and are involved in the signal transduction of pain or taste. These channels are gated by increases in $[H^+]_e$, and their activation stimulates conductance of K^+, Na^+, and/or Ca^{2+}, which triggers action potentials (Fig. 1). There are two major groups of H^+-stimulated sensory channels: transient receptor potential (TRP) channels and acid-sensing ion channels (ASICs).

Polycystic kidney disease 2-like 1(PKD2L1) is a TRP channel stimulated by low pH_e. PKD2L1 is present in taste receptor cells in the tongue, where it transduces sour stimuli (i.e., $pH_e<5$) [21]. PKD2L1 is also present in a discrete population of neurons around the central canal of the spinal cord, which respond to minute pH variations around pH 7.4 [21]. This differential sensitivity to pH_e, as well as its functional expression on cellular membranes, is presumed to be due to tissue-specific association with modifier proteins [21,22]. Additional information can be found in recent reviews on taste receptors [23] and PKD channels [24]. PKD2L1 is also involved in CO_2 sensing; in tongue taste buds, PKD2L1 senses extracellular acidification subsequent to carbonic anhydrase IV-dependent hydration of CO_2 (see below).

A second TRP channel activated by acidic pH_e is the vanilloid receptor-1 (VR1 or TRPV1). At $pH_e<5.9$, TRPV1 channels display increased open probability [25]. The stimulatory mechanism depends on the interaction of protons with two glutamic acid residues in the extracellular loop (reviewed in [26]. Because TRPV1 is also stimulated by capsaicin (the main ingredient of hot chili peppers) and heat [27], this channel is thought to sense burning pain throughout the body [28,29].

Acid-sensing ion channels (ASICs), which are predominantly expressed in neuronal and neuroepithelial tissues [30–33], are activated by acidic $pH_e<7$ [32]. ASICs, like all members of the degenerin family of channels, are more permeable to Na^+, but they also transport Ca^{2+} and K^+ [31,32]. There are several ASIC isoforms and splice variants, and their association into hetero- or homo-multimers may confer specific regulatory and kinetic properties in different tissues [33,34].

In ASICs, gating by protons involves multiple residues in the extracellular domain, most notably carboxyl–carboxylate interactions between amino acids in the acidic pocket. These interactions result in the movement of a distal domain that blocks or unblocks the channel pore (ball and socket joint) [35]. Proton gating may also involve displacing Ca^{2+} ions which stabilize the closed state (reviewed in [36]).

ASICs are known to be important for nociception and taste [30–33], and they are also implicated in touch sensation in hairy skin [37], control of circulation by arterial baroreceptors [38], and pH_e sensing by carotid body glomus cells [39]. In addition, certain ASIC variants are present in the pituitary gland [40], bone [41], and smooth muscle [42] (among others [43]), with proposed roles in endocrine function, mineralization, and cell migration and control of circulation, respectively. However, it is not known if these functions are related to pH_e sensing. Finally, similar to PKD2L1, ASICs can also mediate CO_2 sensing via the associated variation in pH_e [44] (see below).

Intracellular pH (pH_i) sensors

Originally, it was posited that the H^+ and HCO_3^- transporting proteins which restore pH_i would be directly responsible for pH_i sensing. While it is true that a subset of these transporters are allosterically modulated by pH_i (e.g., sodium/hydrogen exchanger (NHE) [45]), their responsiveness is not sufficiently sensitive: pH_i regulation demands finer control than the

observed kinetic responsiveness of NHEs and other pH_i regulatory ion transporting proteins (reviewed in [46]).

One "signaling" pH_i sensor is Pyk2, a member of the focal adhesion kinase family of tyrosine kinases [47]. Pyk2 is directly activated by acidic pH, both in cultured cells and in cell-free systems [48]. Although the exact molecular mechanism for acid activation is not known, it results in increased affinity for Pyk2's substrate ATP (decreased K_m) without changing its V_{max} and is dependent on Ca^{2+} ions in the medium [48]. The initial steps of Pyk2 activation by acidic pH are autophosphorylation of tyrosine 402 [49] followed by a physical association with [48], and phosphorylation of, the tyrosine kinase c-Src [49]. While both c-Src and Pyk2 are activated by acidic pH [50], Pyk2 is considered the pH_i sensor because its activation precedes the other events, and c-Src activation is prevented by dominant-negative Pyk2 or siRNA-mediated inhibition of Pyk2 [48].

One of the consequences of acid stimulation of Pyk2 is NHE3 activation [48], which may be mediated by enhanced NHE3 transcription [51,52] and/or by increasing NHE3 insertion into apical membranes [52]. NHE3 activation enhances H^+ secretion to restore pH_i (reviewed in [49]). The Pyk2/c-Src/NHE3 pathway is thought to be important for the compensation of systemic metabolic acidosis by the renal proximal tubule (reviewed in [46,49]) (Fig. 2). Pyk2 and c-Src also mediate the activation of the Na^+/HCO_3^- cotransporter 1 (NBC1, Slc4a4) in response to intracellular acidification by CO_2 [53]. Thus, Pyk2-mediated responses to acidosis can involve increases in both apical H^+ secretion via NHE3 and basolateral HCO_3^- absorption via NBC1 (Fig. 2). Finally, Pyk2 and c-Src are also important for the normal functioning of osteoclasts [54] and endothelial cells [55,56], suggesting their pH_i sensory role extends beyond the kidney (reviewed in [46]).

The renal proximal tubule possesses a pH_i-sensing pathway independent of Pyk2 which leads to ERK activation via an unknown mechanism [49,57]. One candidate for this Pyk2-independent pH_i sensing would be HCO_3^--sensitive soluble adenylyl cyclase (sAC) (described below). The sAC has been shown to be upstream of ERK activation in other cells [58], and it is present in the renal proximal tubule [59]. However, the involvement of sAC in renal ERK activation has not yet been examined.

The pH-sensing protein kinases are also implicated in bacteria. *Salmonella* sense the lower pH inside the mammalian phagosome to stimulate transcription of the genes essential for virulence. In this system, the sensor for lowered pH is kinase PhoQ, which is directly activated by exposure of the sensor domain to pH 5.5 [60]. Also in *Salmonella*, pH_i sensing is important for activation of the machinery necessary for delivering toxin to their host's cells, but in this case, the sensor is not yet known [61].

Bacteria also sense pH_i via second messenger generation. The adenylyl cyclase from *Mycobacterium tuberculosis* (Rv1264) [62] is activated by low pH. The molecular mechanism that confers Rv1264 pH sensitivity depends on the separation of an inhibitory subunit from the catalytic subunit [62]. Although Rv1264 was hypothesized to be important for sensing and counteracting acidification of phagolysosomes during host invasion [62], mutants lacking this cyclase demonstrated normal virulence [63]; no other in vivo role of Rv1264 is known.

The adenylyl cyclases from enteric bacteria (CyaA) such as *Escherichia coli* are also pH sensitive, but they are activated by high pH [64]. Purified CyaA demonstrates a very steep pH-dependency in the physiological range (between pH 7 and 9) in vitro, and while CyaA mediates the well-studied process of catabolite repression (reviewed in [65]), there is no defined role for pH_i sensing in this process. Conversely, it has been postulated that cAMP mediates physiological responses to pH_i changes [66,67], but a definitive role for CyaA has not been established. Even though orthologs of Rv1264 and CyaA have not been found in eukaryotes,

these studies reveal an evolutionarily ancient connection between second messenger signaling and pH_i sensing, and establish the paradigm of nucleotidyl cyclases acting as molecular sensors of pH_i.

Recent reviews about other molecular pH_i-sensing mechanisms can be found in [68] and [69].

Bicarbonate (HCO_3^-) sensors

Inside cells, HCO_3^- is used as a cofactor for carboxylation reactions, particularly via the vitamin K-dependent carboxylases, whose substrates play roles in blood coagulation, apoptosis, bone mineralization, calcium homeostasis, growth control, and signal transduction in the brain [70,71]. However, vitamin K-dependent carboxylase is maximally stimulated at a $[HCO_3^-]<2$ mM [72], which is way below its intracellular level, indicating that their activity would not fluctuate with physiologically relevant changes in bicarbonate. Thus, although they are HCO_3^--utilizing enzymes involved in signaling cascades, they do not satisfy the requirements for functioning as HCO_3^- sensors.

In contrast, two vertebrate signaling enzymes are responsive to physiologically relevant changes in intracellular $[HCO_3^-]$—soluble adenylyl cyclase (sAC, also known as ADCY10 and Sacy) and guanylyl cyclase-D (GC-D) [73–75]—for example, sAC has been demonstrated to be modulated in vivo by $[HCO_3^-]$ which (a) originates via carbonic anhydrase-dependent hydration of exogenous CO_2 [59,75–77], (b) enters the cell via DIDS-sensitive NBCs [Choi et al., unpublished], and (c) is generated from metabolic CO_2 production inside mitochondria [78,79] (Fig. 3).

sAC is directly activated by HCO_3^- to produce the second messenger cAMP [73]. sAC is widely expressed [80–82], and it is distributed throughout the cell's cytoplasm and in organelles such as the nucleus, centriole, mitotic spindle, mid-body, mitochondria [83], and in cilia [84]. sAC orthologs are present from cyanobacteria to mammals, suggesting that HCO_3^- sensing via sAC-like cyclases is evolutionarily conserved [73,85].

A mechanistic model for cAMP formation from ATP by sAC-like enzymes has been deduced from the HCO_3^--regulated cyanobacterial sAC homolog CyaC [86], which is both structurally and kinetically similar to mammalian sAC [86,87]. Like other class III adenylyl cyclases, sAC requires two divalent cations for activity [88,89]. Though active with Mg^{2+} as the only available divalent ion, addition of Ca^{2+} increases the affinity (decreases K_m) for its substrate ATP to values consistent with the concentration of ATP found inside the cells [90]. These data suggest that in vivo mammalian sAC utilizes both Mg^{2+} and Ca^{2+}, and that its activity will be sensitive to ATP fluctuations inside the cells. The structure- and kinetics-based model predicts that Ca^{2+} bound to the γ-phosphate of ATP enters the catalytic site and coordinates with specific residues in the sAC catalytic center, resulting in an "open sAC state". The second divalent metal, in this case a Mg^{2+} ion, then binds to the α-phosphate of ATP, leading to a "closed state" by interaction with a different set of catalytic residues. The change from "open" to "closed" states induces the release of the β- and γ-phosphates and esterification of the remaining alpha phosphate with C3 of the ribose in adenosine ("cyclizing"). HCO_3^- stimulates substrate turnover (V_{max}) by inducing an allosteric change which leads to active site closure, recruitment of the catalytic Mg^{2+}, and rearrangement of the phosphates in the bound ATP, thus facilitating cAMP formation and release [87].

There is a single functional sAC gene in the human genome; however, it utilizes multiple promoters, and sAC mRNA undergoes extensive alternative splicing [80,81,84,91]. Full-length mammalian sAC (sAC_{fl}) is comprised of two heterologous catalytic domains (C1 and C2) which constitute the amino terminal 50 KDa of protein. The additional ~140-kDa C terminus

of sAC_{fl} includes several putative regulatory domains such as an autoinhibitory region [92] and canonical P-loop and leucine zipper sequences [85] of as yet unknown functions. The minimal functional sAC variant, termed sAC_t, is a truncated form almost exclusively comprised of C1 and C2 [85,91]. This C1C2 protein has robust intrinsic basal and HCO_3^--stimulated activities, and it is sensitive to all known sAC inhibitors. Other sAC variants have not yet been characterized in detail, but molecular studies predict the existence of C2-only isoforms resulting from alternative splicing and promoter utilization [80,81,84]. Existence of these C2 isoforms provide a rationale to explain why male infertility is the only reported phenotype of the existing sAC-knockout mouse model (which deleted exons encoding the C1 domain) [93–95]; this sAC-C1 knockout mouse seems to retain the alternative promoter generating the putative C2-only proteins [80]. Confirmation of this hypothesis awaits generation of KO mice which specifically disrupt the C2 domain.

For mammalian sAC, HCO_3^- half-maximal effect (EC_{50}) ranges between 10 and 25 mM [73,90], which is roughly equivalent to the $[HCO_3^-]$ levels found inside the cells and in plasma. In contrast, the HCO_3^- EC_{50} for sAC from the dogfish shark is ~5 mM [76], reflecting the lower $[HCO_3^-]$ of the internal fluids of aquatic animals compared to air breathers. It is thus possible that the kinetics of HCO_3^- activation will be specific for each sAC ortholog so they reflect the characteristics of that species physiology.

sAC serves as a sensor of extracellular pH/HCO_3^- in the acid/base regulatory epithelia of the mammalian epididymis [59,96] and kidney collecting duct [97,98], and in shark gills [76]. Despite the evolutionary and physiological diversity of these epithelia, the sAC-dependent mechanisms for sensing and regulating pH and $[HCO_3^-]$ in extracellular fluids are astonishingly similar (Fig. 4). In each case, the extracellular stimulus (i.e., elevated luminal pH or $[HCO_3^-]$) is transmitted inside of the cell either by HCO_3^- movement through anion transporters [99] or by entrance of CO_2 via diffusion (or by facilitated diffusion through aquaporins [76,99,100]). When CO_2 diffusion into the cell is the initiating signal, extracellular carbonic anhydrase IV presumably functions to rapidly equilibrate increases in $[HCO_3^-]_e$ into readily available CO_2 [77,99], which can diffuse into the cell. A second carbonic anhydrase (type II) inside the cell would be essential for hydrating CO_2 back into H^+ and HCO_3^- [59, 77,99].

Regardless of how the bicarbonate enters the cells, in these pH/HCO_3^- sensing epithelia, the elevated $[HCO_3]_i$ stimulates sAC to produce cAMP, which activates protein kinase A (PKA) [96,98]. The end effect is translocation of vacuolar proton pump (V-ATPase)-containing vesicles into the cell membrane facing the increase in pH_e/$[HCO_3^-]_e$. In the clear cells of the epididymis and in renal A-type cells, the sAC-dependent V-ATPase translocation is into the apical membrane, producing the elongation of apical microvilli [59,98,101], while in shark gill base-secreting cells, the sAC-dependent V-ATPase translocation is into the basolateral membrane [76,77,102,103]. The transport of H^+ to the side with the original alkalotic stimulus not only counteracts the original alkalosis but it also supplies the H^+ which can combine with the elevated HCO_3^- to generate the CO_2 which fuels the cycle. In the whole animal experiments in the shark, this cellular mechanism was demonstrated to be relevant for systemic pH regulation. The components of this self-regulating loop, sAC, carbonic anhydrase, and V-ATPase are evolutionarily ancient enzymes, suggesting that sensing and regulation of pH/$[HCO_3^-]$ via this signaling module is likely to be conserved. Variations of this mechanism due to differential CO_2 permeability, cell polarization, and association with other sensors and/or downstream effector proteins could result in additional homeostatic mechanisms for CO_2/HCO_3^-/pH.

Bicarbonate sensing via sAC is not limited to systemic acid/base regulation. sAC activity was originally detected in mammalian testis [104,105], specifically in male germ cells. A

biochemically related but particulate activity was detected in spermatozoa [104], and its activity was postulated to be downstream of a $NaHCO_3$ signal [106–109]. Once the sAC gene was cloned [85], it was confirmed that sAC isoforms are highly expressed in testis and sperm [82]. Prior to ejaculation, sperm are stored in the lumen of the epididymis, where [HCO_3^-] is much lower than in plasma (≤5 mM) [110]. Sperm are immotile and incompetent to fertilize an egg while in the epididymis, but upon ejaculation, sperm are mixed with prostatic and seminal fluids containing 25 mM HCO_3^-, which enters sperm via Na^+/HCO_3^- cotransporters [111]. The HCO_3^- influx induces two stages of sperm activation essential for fertilization, motility, and capacitation [112–114], which were pharmacologically [95] and genetically [93–95,115] confirmed to be due to sAC. Thus, sAC functions as a physiological bicarbonate sensor in mammalian sperm essential for fertilization.

In recent years, HCO_3^- sensing via sAC has also been associated with the regulation of NaCl absorption in mammalian kidney [116] and fish intestine [117]; cystic fibrosis transmembrane conductance regulator (CFTR) in human airways [118,119] and bovine cornea [120]; osteoclast formation [121], early embryo development [122], and K^+ secretion in colon [123]; and fluid secretion in cholangiocytes [124] (Table 1).

Like sAC, GC-D is directly activated by HCO_3^-, but GC-D produces the second messenger cGMP instead of cAMP [74,75]. The EC_{50} of GC-D for [HCO_3^-] is ~20 mM, both in intracellular cGMP accumulation experiments in cells overexpressing GC-D and in cGMP production assays with purified recombinant GC-D catalytic domain [74,75]. In rodents, GC-D is present primarily, if not exclusively, in olfactory neurons [125], and it appears to be a pseudogene in primates [126].The proposed function for GC-D is as a putative sensor of atmospheric CO_2 in rodents [75,127]; however, it is difficult to rectify how an enzyme whose EC_{50} for [HCO_3^-] corresponds to ~4% CO_2 could sense changes in atmospheric CO_2 (~0.03%).

Carbon dioxide (CO_2) sensors

In insects and nematodes, CO_2 sensing induces either attraction or avoidance depending on the species and the particular neurons involved (recently reviewed in [128]). In CO_2-sensing insects, like the malaria-carrying *Anopheles* mosquito and the fruit fly *Drosophila*, gaseous CO_2 is sensed by chemosensing neurons of the olfactory system [129]. Drosophila also sense CO_2 in solution, but this is mediated by different neurons of the gustatory system [130]. In fruit flies and the mosquito, two seven-transmembrane spanning members of the gustatory receptor family (Gr21A and Gr63A) are essential for sensing volatile CO_2 [131]; however, the exact molecular mechanisms, including whether they sense CO_2, HCO_3^-, or H^+, and their downstream signaling cascade(s), remain unknown (reviewed in [128]).

In the nematode *Caenorhabditis elegans*, CO_2 induces an avoidance behavior mediated via cGMP [132,133]. The avoidance response is not induced by acidic pH or elevated [HCO_3^-] in media, leading the authors to suggest that the stimulus is a change in the concentration of H^+, HCO_3^- and/or CO_2 inside sensing cells [132]. The receptor guanylyl cyclase DAF-11 [133] as well as the cyclic nucleotide-gated channel subunits TAX-2 and TAX-4 [132,133] are essential for *C. elegans* avoidance behavior and establish the link to cGMP. However, DAF-11 does not seem to be present in the same neurons as TAX-2 and TAX-4 [134], which possibly indicates a complex system with more than one chemoreceptor located in several sensing neurons.

In mammals, CO_2 is sensed in kidney, airways, tongue, and peripheral and central chemoreceptors. Although some prokaryote adenylyl cyclases may be directly activated by CO_2 [135], a similar regulation has not yet been unambiguously identified in mammals. In part, this may be due to the difficulties associated with differentiating between direct effects of CO_2 from effects due to pH and HCO_3^-. The use of out-of-equilibrium CO_2/HCO_3^-

solutions [136] is one of the few ways in which this issue can be addressed. Using this approach, sensitivity to pharmacological inhibitors implicates tyrosine kinases in promoting acid secretion/bicarbonate reabsorption in response to basolateral ("blood") CO_2 in kidney proximal tubules [137,138]. Interestingly, these experiments conclude that the inducing signal is CO_2/HCO_3^- and not pH_i, which suggest involvement of an as yet undefined CO_2/HCO_3^- sensor instead of being mediated by Pyk2 and c-Src, which are proposed to function in the proximal tubules to activate apical H^+ secretion by NHE3 in response to acidosis [46,48,49].

Because of the rapid equilibration of CO_2, HCO_3^-, and H^+ by carbonic anhydrases, in multiple physiological systems, CO_2 is sensed by a pH sensor or a HCO_3^- sensor coupled with a carbonic anhydrase, for example, as mentioned above, the mechanism for extracellular CO_2 sensing in sour taste receptor cells depends on extracellular carbonic anhydrase IV and PKD2L1 [139], the polycystickidney-disease-like acid-selective ion channel [21,22]. Carbonic anhydrase IV hydrates CO_2 into HCO_3^- and H^+ almost instantaneously; the elevated $[H^+]$ opens PKD2L1 channels, which allow the entry of cations into the cell and elicit the neuronal response [139] (Fig. 1). Extracellular CO_2 is also sensed via related variations in pH_e by acid-sensing ion channels (ASICs)[1] in the amygdala, and this pathway is important in fear-induced behaviors [44].

In vertebrates, peripheral chemoreceptors, specifically the aortic and carotid bodies, sense changes in arterial CO_2 and pH while central chemoreceptors sense CO_2 and pH of cerebral spinal fluid, and both types of chemoreceptors regulate breathing frequency and tidal volume. The chemosensing neurons may sense CO_2, $[HCO_3^-]$, pH_e, pH_i, transmembrane pH gradient, or oxidative stress, with the ultimate response possibly depending upon a combination of multiple sensors [140]. Although the potential direct effect of CO_2/H^+ on ion channels has been extensively studied ([141–144], see previous section on H^+ sensors), no molecular chemosensor has been unambiguously identified. Bicarbonate-sensitive sAC represents an additional intriguing candidate which has not yet been directly tested. sAC is present in carotid body [145], and cAMP levels in carotid body are elevated during hypercapnia [145,146], reviewed in [140]. sAC was hypothesized to sense intracellular increases in $[HCO_3^-]$ derived from hypercapnia in type I (glomus) carotid body cells and to augment Ca^{2+} influx through L-type Ca^{2+} channels via PKA [146]. This stimulation is independent of changes in pH_e or pH_i [146] and, like the CO_2 sensing mechanisms explained above, probably depends on a carbonic anhydrase (c.f.[147]). We have confirmed the presence of sAC in rat glomus cells by immunofluorescence (unpublished observations), which also express abundant intracellular carbonic anhydrase [148,149]. Additionally, L-type Ca^{2+} channels are activated by elevated CO_2, independently of pH_i, in rat locus coeruleus neurons [143], suggesting sAC could also be important in central chemosensing; however, direct functional evidence for the role of sAC as a molecular sensor of hypercapnia in the carotid body or in other peripheral and central chemoreceptors, is lacking.

It has already been demonstrated that bicarbonate-regulated adenylyl cyclases, in conjunction with carbonic anhydrases, can function as CO_2 sensors. In the pathological fungi *Candida albicans* and *Cryptococcus neoformans*, the adenylyl cyclases (Cyr1) are essential for pathological differentiation. In both cases, the ACs are directly regulated by HCO_3^- [150, 151], and deletion of either the cyclase or the carbonic anhydrase abrogates pathogenicity. These pathogenic fungi know when they are inside an infected host, and hence when they should undergo their pathogenic differentiation, by sensing the host's internal $[CO_2]$ (5%), which is 150-fold higher than the ambient $[CO_2]_e$ (0.03%).

[1]In this case, it is not known whether the mechanism relies on the presence of a carbonic anhydrase.

In mammals, CO_2 chemosensing via sAC has been demonstrated in the regulation of ciliary beat frequency (CBF) in lung epithelial cells. CO_2 exposure increases CBF in cultured human lung epithelial cells differentiated at an air-liquid interface. This response is mediated by CO_2/ HCO_3^--dependent stimulation of sAC localized to the cilia's axoneme [84]. Carbonic anhydrases are present in the apical area of ciliated cells of bronchiolar epithelium [152], but a direct link of carbonic anhydrases to CBF has not yet been established. Analogy with other CO_2-sensing mechanisms suggests that carbonic anhydrases mediate the stimulation of sAC in cilia. Because CO_2 levels are higher in exhaled breath compared to inhaled air, the regulation of CBF by sAC may ensure the proper clearance of mucus from airway epithelia, and impairments in this regulation may be associated with airway diseases such as asthma [84, 153].

Carbonic anhydrase and sAC also sense metabolically generated CO_2 inside mitochondria. Mitochondria are the predominant source of CO_2 in eukaryotic cells, and sAC is present inside mitochondria [83], where it coordinates the rate of ATP production via oxidative phosphorylation with nutritional availability [78,79]. Mitochondrial sAC activity is stimulated by Krebs Cycle-generated CO_2 in a carbonic anhydrase-dependent manner. CO_2/HCO_3^- stimulation of sAC activates intramitochondrial PKA which phosphorylates complex IV of the electron transport chain, increasing its rate and capacity to handle electrons. Thus, a mitochondrial CO_2–carbonic anhydrase–sAC pathway couples nutritional status to ATP production to ensure minimal generation of reactive oxygen species, and sAC senses variations of intracellular (metabolic) changes in CO_2/HCO_3^- in addition to $[HCO_3^-]_i$ secondary to extracellular (blood, environment) CO_2, HCO_3^- and pH changes.

Conclusions

Biological systems sense CO_2, HCO_3^-, and pH via multiple different types of sensors, and while the specifics may vary, the fundamentals of sensing are evolutionarily conserved. HCO_3 and pH are directly sensed by protein kinases and nucleotydyl cyclases from bacteria (e.g., CyaA, PhoQ) through vertebrates (e.g., sAC, Pyk2), and CO_2 is indirectly sensed by HCO_3^- or pH sensors coupled to carbonic anhydrases. Thus, mechanisms for sensing pH, $[HCO_3^-]$, and CO_2 are present in all domains of life illustrating their importance for organismal function.

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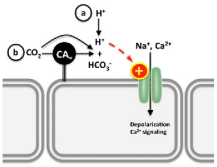

Fig. 1.

Extracellular pH/CO_2 sensing by H^+-gated channels in sensory neurons. *a* Extracellular H^+, which could derive from elevated CO_2 (*b*), stimulate cation currents. Examples include TRP channels, such as PKD2L1 and TRPV1, and ASICs. If coexpressed with extracellular carbonic anhydrase IV (CA_{IV}), these channels can indirectly sense extracellular CO_2

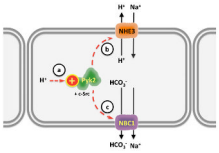

Fig. 2.

Intracellular pH sensing by the tyrosine kinase Pyk2 in the renal proximal tubule. *a* Intracellular H^+ activate PyK2 and c-Src, which promote apical H^+ secretion and Na^+ absorption by *b* sodium hydrogen exchanger 3 (*NHE3*) and *c* Na^+ and HCO_3^- absorption by sodium bicarbonate cotransporter (*NBC*)

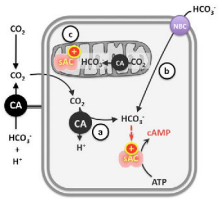

Fig. 3.

Activation of sAC inside the cells. Production of cAMP by sAC can be modulated by HCO_3^- which *a* originates via carbonic anhydrase (*CA*) hydration of exogenous CO_2, *b* enters the cell via sodium bicarbonate cotransporter (*NBC*), or *c* is generated from metabolic CO_2 production inside mitochondria

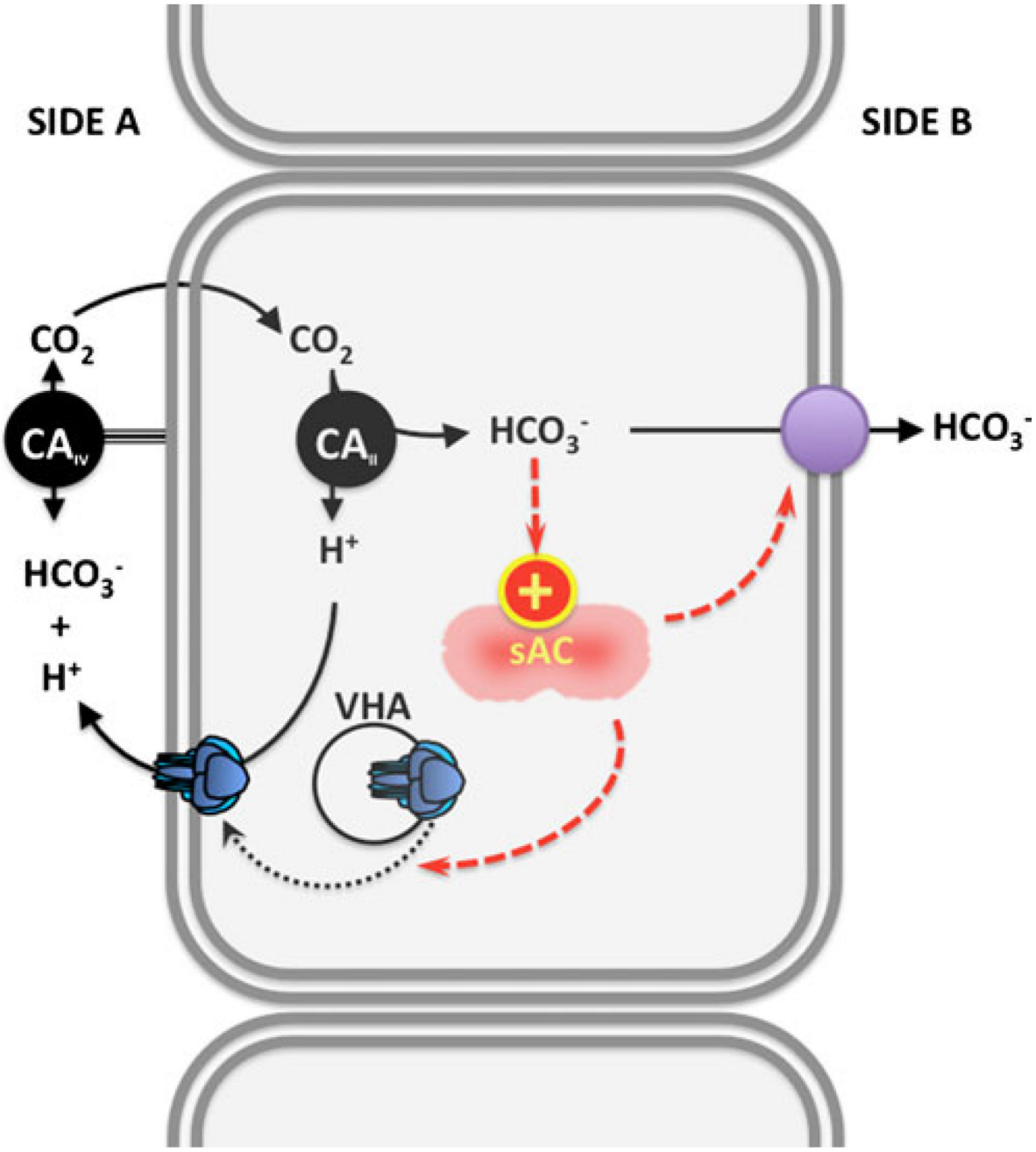

Fig. 4.

Sensing and regulation of systemic pH and acid/base status by carbonic anhydrase (*CA*), sAC, and vacuolar proton pump (*VHA*). Alkalosis due to elevated extracellular HCO_3^- and/or pH results in elevated extracellular CO_2, a reaction catalyzed by extracellular CA_{IV}. CO_2 diffuses inside the cell, where it is hydrated into H^+ and HCO_3^-. sAC is activated by intracellular HCO_3^- to produce cAMP, which promotes (via PKA) the insertion of VHA-containing vesicles into the cell membrane facing the alkalosis. Membrane-inserted VHAs secrete H^+, which counteract the alkalosis. sAC may also modulate the activity of HCO_3^- transporters in the opposite membrane. In the clear cells of the epididymis and in A-type renal intercalated cells,

Side A is the apical (mucosal) side and *Side B* is the basolateral (serosal) side. The polarity is reversed in base-secreting cells of the shark gill and in B-type renal intercalated cells

Table 1

Putative CO_2/pH/HCO_3^- sensing via sAC

Function	
pH and [HCO_3^-] sensing	
Acid/base and pH regulation	Regulation of V-ATPase recycling/translocation to the cell membrane in clear cells of the epididymis [59,96], A-type intercalated cells of mammalian kidney [97,98,101], and base-secreting cells in shark gill [76,77].
Ion and fluid transport	NaCl absorption in renal collecting duct [116] and toadfish intestine [117], HCO_3^- transport in epithelial airways [118], Cl^- secretion in cornea [120], K^+ secretion in colon [123], and fluid and Cl^- secretion in pancreas [124].
Regulation of gene transcription	CREB phosphorylation [154]; CFTR gene transcription [119].
Sperm function	Sperm motility, capacitation, and possibly acrosome reaction [93–95,115,155].
Brain metabolic coupling	Glycogen breakdown and lactate release by astrocytes to be used by neurons [Choi HB, Gordon GRJ, Zhou N, Tai C, Ryu JK, McLarnon JG, Levin LR, Buck J, MacVicar BA (2010) Metabolic communication between astrocytes and neurons via bicarbonate responsive soluble adenylyl cyclase. unpublished].
Development and cell differentiation	Osteoclastogenesis [121]; early embryo development [122].
CO_2 sensing	
Cilia movement	Cilliary beat frequency in airway epithelia [84].
Metabolic regulation	Mitochondrial oxidative phosphorylation in response to CO_2 from TCA cycle [78,79].