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Sensing, physiological effects and molecular response to elevated CO_2 levels in eukaryotes

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Abstract

Carbon dioxide (CO_2) is an important gaseous molecule that maintains biosphere homeostasis and is an important cellular signalling molecule in all organisms. The transport of CO_2 through membranes has fundamental roles in most basic aspects of life in both plants and animals. There is a growing interest in understanding how CO_2 is transported into cells, how it is sensed by neurons and other cell types and in understanding the physiological and molecular consequences of elevated CO_2 levels (hypercapnia) at the cell and organism levels. Human pulmonary diseases and model organisms such as fungi, *C. elegans*, *Drosophila* and mice have been proven to be important in understanding of the mechanisms of CO_2 sensing and response.

Keywords: CO_2 • hypercapnia • lungs • *D. melanogaster* • *C. elegans* neuronal chemosensors • soluble adenylate cyclase • signal transduction

CO_2 transport into cells

Transport of carbon dioxide (CO_2) through membranes has fundamental roles in many aspects of life including photosynthesis, nutritive transport, oxidative metabolism and signalling. Once transported into the cell, CO_2 interacts with H_2O to produce carbonic acid, which is in equilibrium with H^+ and HCO_3^-. At 37°C, this reaction is accelerated 0.5 to 1 $\times$ 10^6 times by the zinc-containing enzyme carbonic anhydrase (CA). Another chemical reaction affecting CO_2 concentration within cells is its interaction with a free R-NH^{3+} group on proteins to form carbamate. Although work presented below suggests that there are specific transporters that help move CO_2 across membranes, a recent study supports the hypothesis that passive diffusion is sufficient to provide

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necessary transport and that facilitated transport of CO_2 is not necessary. This study measured the changes in CO_2 gradients through planar lipid bilayer membranes and through epithelial cell monolayers. Membrane permeability was calculated from measuring small changes in pH and by applying Overton's rule, which in lay terms, dictates that the easier it is for a chemical to dissolve in a lipid the faster it will be transported into a cell. This study concluded that passive diffusion could explain CO_2 transport into cells with a rate-limiting step of near-membrane unstirred layer [1]. It should be noted, however, that a recent study questions the validity of Overton's rule and thus the sufficiency of passive diffusion of CO_2 [2].

The hypothesis that CO_2 is transported solely by passive diffusion through membranes has been challenged by several observations including the impermeability of apical membranes of gastric gland cells to CO_2 [3], the reduction of permeability of red blood cells (RBCs) to CO_2 by the anion transport inhibitor 4,4′-Diisothiocyanatostilbene-2,2′-disulfonate [4], and by the discovery of evolutionarily conserved proteins that can serve as CO_2 transporters [5–8].

One of the major conserved CO_2 transporter-protein families are the aquaporins (AQP). In plants, CO_2 uptake can be a rate-limiting step in photosynthesis. Work in the plant *Nicotiana tabacum* showed that the water channel AQP could also serve as a CO_2 channel. Overexpression of the aquaporin AQP1 gene increased membrane permeability for both CO_2 and water, and enhanced leaf growth [5], while down-regulation of AQP1 lowered CO_2 permeability in isolated membranes of the inner chloroplast membrane, where AQP1 resides, and caused a 20% lower conductance to CO_2 within leaves *in vivo* [6].

The rhesus (Rh) protein family is another putative CO_2 channel. Rh proteins are best known as antigens on human RBCs, but they are not restricted to RBCs and are conserved in evolution. In organisms as diverse as human and yeast the Rh proteins function as ammonium and methylammonium transporters [7]. Work in the green alga *Chlamydomonas reinhardtii* showed that the Rh protein, RH1, can also function as a CO_2 channel [8]. Expression of *rh1* is induced under high CO_2 and is suppressed under low CO_2 [9]. Alga, in which the *rh1* gene has been down-regulated *in vivo* grow normally in air, but slowly in high CO_2, apparently because they fail to equilibrate CO_2 rapidly. That Rh1 is important for CO_2 responses is demonstrated by the observation that *Chlamydomonas reinhardtii* genes that are known to be down-regulated by high CO_2 fail to be down-regulated in RH1-null cells [8]. Methylammonium uptake is little changed by the absence of Rh1, suggesting that in *Chlamydomonas reinhardtii* either RH1 does not function as an ammonium channel or that there are other transporters that can handle ammonium transport in the absence of Rh1.

In mammalian cells there is evidence for both AQP and Rh proteins having roles in CO_2 transport. In AQP1-null RBCs (known as Colton null RBCs) CO_2 transport was reduced by 50% [10]. Inhibition of AQP1 in RBCs by the inhibitor p-chloromecuribenzene sulfonic acid reduced pCO_2 by 60%. These results led to the conclusion that AQP1 is responsible for ~60% of the high pCO_2 in RBCs. Band 3 complex in RBCs includes both AQP1 and the Rh proteins [11, 12]. RBCs lacking Rh protein had only 50% transport of CO_2, similar to the reduction in RBCs cells lacking AQP1 [13], suggesting overlapping functions between the AQP and Rh proteins.

It should be noted that in addition to transporting ammonium/methylammonium, CO_2 and H_2O there is evidence that Band 3, Rh and AQP1 complexes can transport O_2 and nitric oxide, which makes them non-specific gas channels [14, 15]. However, although AQP and Rh channels can transport a broad range of gases, they do not transport all gases equally. In a recent study, genes encoding human AQP1, rat AQP4, rat AQP5, human RhAG and bacterial AmtB were injected into *Xenopus* oocytes and then surface pH was measured to establish the relative selectivity to CO_2 and NH_3 [16]. It was found that the different channels have different gas selectivities with the AQP channel more efficiently transporting CO_2, while the Rh channel more efficiently transported NH_3.

How important are the Rh and AQP proteins to animals as a whole? In *C. elegans*, one of the Rh1 proteins (CeRH1) is expressed in all tissues albeit with higher expression in the hypodermis, which is the tissue directly exposed to the surrounding atmosphere. One study showed that mutation of the *rh1* gene causes lethality and abnormal development [17] This study, however, did not assess the role of CeRh1 in adult CO_2 responses as it was only later discovered that elevated CO_2 reduces the number of eggs laid by mothers [18] (see section 'Physiological effects on *C. elegans*'). Another *C. elegans* study tested the effects of homozygous deletions in each of the two Rhesus genes: *rh1* and *rh2*, as well as in the aquaporin gene *aqp-2* on egg-laying in animals grown in 15% or 19% CO_2. They found that for all the single deletions the number of laid eggs was further reduced in CO_2, suggesting that in *C. elegans* the response to hypercapnia is independent of these genes [18].

Fungi, like *C. elegans*, do not require Rh1 or AQPs for CO_2 transport. The counterparts for the Rh proteins are the Mep $NH3/NH4^+$ ion transporters. *S. cerevisiae* and the two *C. albicans* and *C. neoformans* Mep proteins probably do not transport CO_2 (reviewed in [19]). Likewise, the *C. albicans* aquaporin *aqy1* gene is required for freeze-tolerance but not for CO_2-mediated filamentation [20].

In conclusion, despite considerable work in many species, how CO_2 is transported into cells is still a matter of some debate. While passive diffusion through membrane can explain much of the CO_2 transport, the passive diffusion model cannot explain the low permeability of gastric cells and RBCs to CO_2 and the demonstrated roles of AQP and Rh protein complexes in CO_2 transport in plants, some lower organisms and in RBCs. More studies, including simultaneous knockouts of the Rh and AQPs in mice, *D. melanogaster* and *C. elegans* are required to understand how CO_2 is transported across cell membranes.

CO_2 sensing in cells and organisms

Chemoreception, the recognition of soluble and volatile chemicals by cells, plays an essential role in the behaviour and survival of

most organisms. CO_2 is a by-product of metabolism and is an important signal for a variety of animal behaviours, including feeding, ventilation, mating and avoiding predators and harmful substances. The ability to sense CO_2 has been described in many eukaryotes ranging from fungi to human beings.

CO_2 sensing in mammalian neuronal cells

From a physiological standpoint, some of the most important CO_2 sensors are those that control breathing in mammals. Changes in CO_2 and CO_2/H^+ levels are sensed in special chemosensitive neurons located peripherally in the carotid body and centrally in the central nervous system. The peripheral carotid neurons detect arterial CO_2 and pH, as well as variations in O_2 levels in arterial blood [21]. The central neurons reside in several regions of the hindbrain and detect CO_2 and pH in the cerebrospinal fluid [22].

CO_2 sensing in peripheral chemoreceptors

The carotid body (*carotid glomus*) is located near the fork of the carotid artery. It has two types of cells; catecholamine-containing type I (glomus) cells, which are sensitive to changes in both CO_2 and O_2 levels in arterial blood [21, 23], and glial-like type II cells. The type I glomus cells are the primary site of peripheral chemoreception, where they act as a sensor that detects changes in CO_2 and O_2 levels in arterial blood [21, 23]. During normal breathing, the carotid chemoreceptors are critical for maintaining stable and normal CO_2 levels [24]. While the central chemosensitive neurons appear to have a quantitatively larger contribution to stimulating ventilation in response to hypercapnia [25], during mild hypercapnia the carotid chemosensitive neurons contribute to roughly 30% of the response (summarized in Table 1 in [21]). Importantly, the response of the carotid body neurons is quicker than that of the central neurons and the carotid neurons react first to rapid transient changes in arterial CO_2 levels [26]. Indeed, denervation of the carotid body causes a slower response to changes in CO_2 levels and a constant fluctuation in CO_2 levels during normal breathing. Carotid body denervation also decreases normal breathing, causing 13–18 mmHg increase in CO_2 levels in different mammals including awaked dogs, goats and ponies [27–31].

It is not clear yet what the exact signal sensed by the glomus cells is or what the molecular sensor is. Studies have suggested that it could be elevated CO_2 levels, HCO_3^- concentration, reduced intracellular pH or a pH gradient across the membrane. For example, inhibition of intracellular change in pH by using a permeable CA inhibitor slowed down the firing of the neurons, suggesting that change in pH is important for the response of these cells [22, 32]. On the other hand, hypercapnia-induced acidosis had much greater effect on neuron firing as compared to metabolic acidosis [33], suggesting that CO_2 or changes in pH across the membrane are the signals for neuron firing [34]. While the identity of the CO_2 sensor is not clear, it has been determined that neuronal firing in response to CO_2 requires L-type Ca^{2+} channels, protein kinase A (PKA) and soluble adenylate cyclase (sAC) (see section 'Molecular responses in mammalian neuronal cells').

CO_2 sensing in central chemoreceptors

The central chemosensitive neurons have a major contribution in mediating increased ventilation in response to hypercapnia. These neurons are found in numerous hindbrain stem regions and have different levels of chemosensitivities. They include the retrotrapezoid nucleus (RTN), the rostral medullary raphe, the caudal nucleus tractus solitarius, the fastigial nucleus of the cerebellum, locus ceruleus, A5 region and the pre-Bötzinger complex [35]. Further, *in vitro* and *in vivo* studies have shown that additional types of neurons can respond to changes in CO_2 levels. These neurons show altered firing rate in response to changes in CO_2 levels and reside in regions shown to alter ventilation in response to acidification. However, it is not clear yet whether they intrinsically response to changes in CO_2 levels or whether they respond to altered synaptic input from other neurons that are themselves chemosensitive (reviewed in [22]).

The central chemoreceptors detect changes in CO_2/H^+ of cerebralspinal fluid, which are caused by changes in CO_2 that diffuse from the plasma to the cerebrospinal fluid and forms carbonic acid [36]. To avoid a potential general inhibitory effect by CO_2/H^+, most of the studies on central chemosensitive neurons have focused on CO_2-excited neurons [37]. However, in studies on slices from the dorsal or ventral medulla, half of the neurons were actually inhibited by hypercapnia [38, 39], suggesting that many features of hypercapnia neuronal responses are missed when studying only CO_2-excited neurons. Rat brain stem slices were also used to show the specificity of chemosensitive neurons [40, 41]. Upon exposure to hypercapnic acidosis, neurons from the chemosensitive ventrolateral medulla and the nucleus tractus solitarii regions became acidic and remained acidic during the entire exposure, with pH returning to control values upon return to normocapnic solution. In contrast, neurons from the non-chemosensitive inferior olive and hypoglossal regions recovered from the hypercapnia acidosis-induced acidification during the period of acid exposure [42].

Similar to what has been described for the carotid body, it is not clear whether the central chemosensitive neurons detect pH levels, CO_2 levels, HCO_3^- concentration or a pH gradient across the cell membrane. What is clear is that the ventilatory response to hypercapnia is greater than to metabolic acidosis [43]. In addition, central neurons have greater firing response to hypercapnic acidosis than isocapnic acidosis, where the CO_2 levels remain constant and HCO_3^- concentration and pH are decreased [44, 45]. These observations suggest the existence of CO_2 receptors, analogous to receptors for other gases such as O_2, nitric oxide and CO [46–49]. It was demonstrated that the L-type Ca^{2+} channels mediate responses of CO_2 receptors, whose identity has not been determined (see details in section 'Molecular responses in mammalian neuronal cells'). Another important observation is that there is variability in the chemosensitivity of neurons in different areas of the brain, suggesting different patterns of responses by

the different brain areas. In addition, it is likely that there is not a single adequate stimulus for central chemosensitive neurons but rather multiple stimuli responses and targets.

Key insights into the identities of central neurons required for normal physiological regulation of CO_2 levels has come from studies of genetic diseases and brain lesions. The rare congenital hypoventilation syndrome (CCHS) is defined by the failure of automatic control of breathing. It is characterized by abnormal control of respiration in the absence of neuromuscular or lung disease, or an identifiable brainstem lesion [50, 51]. The patients have absent or negligible ventilatory sensitivity to hypercapnia and hypoxemia. They breathe normally while awake but hypoventilate with normal respiratory rates and shallow breathing during sleep. Severely affected patients hypoventilate both awake and asleep (reviewed in [52]). Most CCHS patients have mutations in the paired-like homeobox gene *PHOX2B* [53, 54]. However, in rare cases this disease can be also caused by mutations in the *RET*, *GDNF*, *EDN3*, *BDNF* and *ASCL1* genes (see OMIM # 209880). The RTN expresses PHOX2B. It contains ~2000 neurons that selectively innervate the respiratory centres of the pontomedullary region in a chemosensitive region [52]. These neurons are highly sensitive to acidic pH *in vitro* and are activated by inputs from the carotid body and from the hypothalamus *in vivo* [52]. Mice heterozygous for the CCHS-causing expanded alanine tract in the *Phox2b* gene show irregular breathing, do not respond to an increase in CO_2, and die from central apnea soon after birth [55]. Postmortem examination of these mice showed specific loss of Phox2b-expressing glutamatergic neurons in the RTN region, whereas other areas thought to be involved in breathing regulation were anatomically normal. In addition, destruction of 70% of RTN neurons helped demonstrating the role of these neurons in normal breathing in anesthetized rats [56]. These data suggest that RTN neurons regulate CO_2 levels by automatic breathing during sleep and also contribute to breathing when awake. Because most patients can breathe at normal rhythm when awake, these data also suggest that the RTN neurons do not directly control breathing rhythm, but rather generate a large portion of the excitatory drive to the centre that controls breathing rhythm.

It still remains to be determined whether the RTN neurons have intrinsic response to CO_2/H^+ *in vivo*. Also, the precise targets of RTN neurons and the modes of synaptic transmission remain to be defined.

CO_2 sensing by non-neuronal mammalian cells

In addition to central and peripheral neurons, other mammalian cell types have also been shown to respond to CO_2. Given the critical role of kidney tubule cells in HCO_3^- homeostasis (see section 'Physiological effects on mammalian tissues'), one might expect kidney cells to be CO_2 responsive. Indeed, work by Zhou *et al.* has provided evidence that there are CO_2 and/or HCO_3^- sensors on the basolateral surfaces of rabbit proximal tubule epithelial cells that regulate HCO_3^- generation and reabsorption [57]. Further work using small molecule inhibitors has indicated that CO_2-induced responses require an as yet unidentified receptor tyrosine kinase that could be the actual CO_2 sensor [58].

There is also considerable evidence that cardiovascular and pulmonary cells respond to CO_2 levels independently of neuronal input. Work in our laboratories has demonstrated that CO_2 levels control cell-surface levels of the Na,K-ATPase in cultured alveolar epithelial cells *via* a CO_2 response pathway that acts independently of extracellular and intracellular pH. This pathway involves Ca^{2+}, Ca^{2+}-calmodulin dependent protein kinase β, AMP-kinase and protein kinase C-ζ (PKC-ζ) (see section 'Lung cells' and Fig. 3) [59, 60]. Other *in vitro* studies have shown that elevated CO_2 levels suppress expression of tumour necrosis factor and other cytokines by pulmonary artery endothelial cells [61] and in peritoneal and pulmonary macrophages [62, 63]. The molecular mechanisms of this suppression are not yet clear, but at least for endothelial cells are thought to involve pH-independent suppression of NF-κB activation [61].

CO_2 sensing in *Drosophila* and other insects

Neuronal-mediated CO_2 sensing

Many arthropods have anatomical features dedicated to CO_2 sensing (reviewed in [64]). Between species, these structures differ greatly at all levels of organization, but all of them are types of olfactory sensilla that can in some cases form specialized CO_2-sensing organs. Insects, and *Drosophila* in particular, have been useful models for the study of chemoreception because there is notable functional and anatomical similarity in smell and taste pathways between vertebrates and *Drosophila* (reviewed in [65]). In some herbivorous adult arthropods, such as beetles and grasshoppers, no CO_2-sensing structures have been identified. Indeed, these structures are most commonly found in blood-feeding insects such as mosquitoes, which presumably use CO_2 as a signal to locate prey [66].

D. melanogaster are able to detect both gaseous CO_2 [67, 68] and CO_2 in water [69]. This distinction serves to illustrate the division of *D. melanogaster* chemoreception into two separate modalities – the detection of volatile molecules (olfaction or smell) and the detection of soluble molecules (gustation or taste). Thus, the fruit fly can 'smell' CO_2 in air, and 'taste' carbonation.

In adult flies, the olfactory sensilla are located on two appendages on the fly head: the maxillary palp and the third segment of the fly antenna [70]. The antenna contains the olfactory receptor neurons (ORNs), organized morphologically into three sensilla used for smell: the basiconic, trichoid and coeloconic sensilla [71]. In contrast, several locations on the fly body contain gustatory sensilla, including the labial palps of the proboscis, sense organs inside the pharynx and taste bristles located on the legs and anterior wing margin.

The antennal basiconic (ab) sensilla can be grouped into three types (ab1, ab2, ab3) based on their odour responses, and the ab1 sensillum contains four different types of ORNs [72]. The sensilla protrude from the antennal cuticle, making electrophysiological measurements relatively easy. In an electrophysiological analysis

of odour coding of these ORNs, the C neuron of the ab1 sensillum (ab1C) was found to respond specifically and strongly to CO_2. These findings were confirmed in a separate study [73].

In *D. melanogaster* adults, gaseous CO_2 elicits an avoidance behaviour [74]. This discovery was made after it was observed that flies preferred to avoid containers in which other flies had previously been exposed to stressful conditions, such as vigorous shaking or electric shock. This behaviour was inhibited by surgical removal of the third antennal segment, indicating that the stressed flies may have emitted a 'stress gas' that was detected by other flies new to the container. Mass spectrometry and gas chromatography revealed that CO_2 was a component of the 'stress gas', and CO_2 alone could elicit the avoidance behaviour in a T-maze assay. This behaviour was dose dependent, with flies avoiding CO_2 concentrations of 0.1% and above. Further, stressed flies were shown to emit 3- to 4-fold more CO_2 than unstressed flies.

This study also found that CO_2 (0.05% and above) causes activation of a single glomerulus, the V glomerulus, in the fly antennal brain lobe [74]. This glomerulus is innervated by ORNs that had previously been shown to express the candidate gustatory receptor Gr21a [75], and which project specifically from the region of the antenna where the ab1 sensilla are located. Subsequent experiments showed that CO_2 activates these Gr21a-expressing neurons, and that they are required for the CO_2 avoidance behaviour. These findings were corroborated by Faucher *et al.* [76]. The importance of Gr21a-expressing neurons to CO_2 responsiveness has been further demonstrated by the finding that in larvae, Gr21a is expressed in a single neuron in the terminal organ, with genetic ablation of this neuron resulting in loss of CO_2 avoidance.

That Gr21a-expressing neurons respond to CO_2 suggests that Gr21a could in fact be a receptor for CO_2. As detailed in section 'Molecular responses in *D. melanogaster*', recent molecular work presents strong evidence that Gr21a, along with the related Gr63a is a receptor is a CO_2 receptor in the *Drosophila* olfactory system [67, 68] see section 'Molecular responses in *D. melanogaster*').

In contrast to gaseous CO_2, which is repellent, carbonation is attractive to *D. melanogaster* adults [69]. The E409 neurons in the gustatory sensilla of the fly proboscis are responsive to both beer and the supernatant of growing yeast, but not to over 50 other tested compounds [69]. After testing two common products secreted by yeast, CO_2 and ethanol, it was found that the neurons could detect carbonation specifically and robustly from 0.2% upwards. However, E409 neurons are also able to respond to high levels of gaseous CO_2, with 100% CO_2 gas causing a response equal to 0.4% CO_2 in water, which is a saturating stimulus. Although the molecular mechanism of gustatory detection of carbonation are not yet known, detection of carbonation is likely not dependent on pH because these neurons do not respond to acids or solutions of varying pH, and pH does not change the attractiveness of PBS solutions to flies. It is possible, however, that the neurons could be detecting carbonic acid. The ability to differentially detect gaseous and aqueous CO_2 may allow the fly to appropriately fine-tune responses to local and global CO_2 levels, or to distinguish between a danger signal and a good meal at the pub.

Non-neuronal CO_2 sensing

As CO_2 is an important environmental cue, one would expect the sensory system to respond to CO_2. One might further expect that because CO_2 is a ubiquitous metabolite, that non-neuronal cells would also have mechanisms for sensing physiological CO_2 levels. The existence of a non-neuronal CO_2-sensing system has recently been demonstrated by showing that in CO_2 levels as low as 7%, both wild-type and adult flies lacking the Gr63a receptor become immune suppressed and lay fewer eggs [77]. Further, 13% CO_2 suppresses the expression of anti-microbial peptides in the immune-responsive S2* cell line. This non-neuronal CO_2 response pathway appears to be a novel response pathway because its activity is independent of pH, nitric oxide, heat shock and hypoxia responses [77]. These results demonstrate that *Drosophila* cells have responses to CO_2 that are independent of the neuronal CO_2 responses. However, it remains to be determined the extent to which neuronal CO_2-sensing impacts responses to CO_2 that can be regulated without neuronal input.

CO_2 sensing in *C. elegans*

Two recent papers have demonstrated that wild-type *C. elegans* (N2) acutely avoid CO_2 [78, 79]. Worms subjected to a 0–5% CO_2 gradient in a microfluidic chamber quickly avoid the area where the CO_2 level is high. Similarly, when the head of a forward-moving worm is exposed to an air stream containing 10% CO_2, within few seconds the animal reversed its direction. In both studies 1% CO_2 was measured as the threshold level of avoidance. This avoidance behaviour is probably independent of any CO_2-induced pH changes, since exposing the animals to different media with pH levels ranging from 4.9 to 7.1 did not affect the avoidance behaviour. cGMP signalling probably contributes to the CO_2 avoidance as *tax-2* and *tax-4* mutants, encoding two subunits of a cGMP gated ion channel, do not avoid CO_2. This result also indicates that TAX-2 and TAX-4 are essential for CO_2 avoidance behaviour. Expression of TAX-4 in BAG neurons alone was sufficient to recover the CO_2 avoidance defect of *tax-4* mutants, thus implicating the involvement of these neurons in CO_2 avoidance and demonstrating the sufficiency of TAX-4 in mediating CO_2-responsive behaviours [78, 79].

Notably, *C. elegans* do not avoid CO_2 in all situations. For example, starved animals do not avoid CO_2, which implicates the involvement of metabolism as a regulator. Indeed, animals with a reduced *daf-2* signalling, which mimics starvation conditions, do not avoid CO_2 [78, 79]. In addition, animals defective in the transforming growth factor (TGF)-β pathway, which is another key regulator of starvation, do not avoid CO_2 [78, 79]. Interestingly, feeding behaviour is intimately linked with CO_2 avoidance behaviour. For example, different wild-type isolates of *C. elegans* show different feeding behaviour. Animals with solitary feeding behaviour strongly avoid CO_2, while animals with social feeding behaviour do not. Genetically interfering with the feeding behaviour can change the way wild-type (N2) animals respond to CO_2. Animals with a null mutation in the *npr-1* gene, a neuropeptide Y receptor, change

their feeding behaviour from solitary to social and accordingly change the way they respond from CO_2 sensitive to CO_2 insensitive. Mutations in *NPR-1* or the neuronal globin domain protein, GLB-5, affect the responses to both CO_2 and O_2, implicating a potential crosstalk between the CO_2 and O_2 response pathways. Genetic variation in these genes is probably responsible for the different responses exhibited by the laboratory wild-type strain N2, which avoids elevated CO_2 but is almost indifferent to O_2 levels, while another wild-type strain CB4856 avoids elevated O_2 levels and is attracted to high CO_2 levels [80].

CO_2 sensing in fungi

Certain fungi pathogenic to human beings sense and adapt to the widely varying CO_2 concentrations of the environments they inhabit, which can have significantly different CO_2 levels. For example, the mammalian blood stream is at ~5% CO_2 which is >100 times higher than ambient air at 0.039%. In *C. neoformans* [81] and *C. albicans* [82, 83] elevated CO_2 levels have strong effects on growth, and induce virulence factors such as capsule biosynthesis and filamentation, (reviewed in [19, 84]). Because virulence factors in these organisms are regulated by adenylyl cyclases and cAMP signalling [85, 86], possible roles for adenylyl cyclases in CO_2 sensing were investigated. Purified adenylyl cyclases from both these species are activated by bicarbonate *in vitro* [20, 87], and appear to act as sensors of elevated CO_2 levels. However, adenylyl cyclases do not act alone in CO_2 sensing in these organisms as CAs, which convert gaseous CO_2 to bicarbonate, were also shown to be involved. The CAs Nce103 of *C. albicans* [20] and Can2 of *C. neoformans* [87] are required for growth and pathogenicity in low CO_2 conditions. However, strains mutant for these CAs can still infect and proliferate in a mammalian host, where CO_2 levels are high and presumably sufficient levels of bicarbonate are produced by spontaneous conversion without the need for catalysis. It is currently unknown how these results generalize to other fungi.

Physiological effects of elevated CO_2

Physiological effects on mammalian tissues

CO_2 is produced by the body's metabolism at approximately the same rate as oxygen consumption (at rest ~3 ml/kg/min.). CO_2 diffuses readily from cells into the bloodstream, where it is carried partly as HCO_3^-, partly in chemical combination with haemoglobin and plasma proteins, and partly in solution at a partial pressure of about 46 mmHg in mixed venous blood. CO_2 is eliminated from the body by the lung, where it is normally exhaled at the same rate at which it is produced, leaving a partial pressure ($paCO_2$) of about 40 mmHg in the alveoli and arterial blood [88].

CO_2 plays a major role in pH homeostasis as part of the CO_2/HCO_3^- buffer system that regulates plasma pH [89]. Unlike the other buffer systems in the body, where addition or loss of hydrogen ions changes the concentration of the weak acid, in the CO_2/HCO_3^- system, the concentration of the weak acid (CO_2) is essentially constant. This is because the $paCO_2$ is regulated by our respiratory system to be about 40 mmHg. Any rise or fall in pCO_2 resulting from the addition or loss of hydrogen ions is sensed by the respiratory centres in the brainstem that alter the rate of ventilation to restore the concentration (see section 'CO_2 sensing in mammalian neuronal cells'). However, adding or removing hydrogen ions from a source other than CO_2 changes the concentration of HCO_3^-. Adding hydrogen ions by diet or some physiological process reduces HCO_3^- on a nearly mole-for-mole basis. Removing hydrogen ions drives the reaction to the right and raises HCO_3^- in the same way. The problem of maintaining hydrogen ion balance becomes one of maintaining HCO_3^- balance. For every hydrogen ion added to the body, one HCO_3^- disappears; therefore, to maintain balance it is necessary to generate new HCO_3^- to replace the one that was lost. Generation of new HCO_3^- is the responsibility of the kidneys. The kidney has two major responsibilities in maintaining acid–base balance. The first is to reabsorb any HCO_3^- that is filtered at the glomerulus and to return it to the plasma. The second is to generate HCO_3^- through non-bicarbonate buffer systems and by metabolizing glutamine [90].

Pathophysiological effects of elevated levels of CO_2

There are several pathological conditions in which the pulmonary, blood or tissue levels of CO_2 increase and cause hypercapnia. Hypercapnia may result from decreased ventilation and non-metabolic generation of CO_2 as occurs during tissue hypoxia. The physiological responses to hypercapnia depend on the concentration of CO_2 and the duration of elevated CO_2 exposure [91]. Low concentrations of CO_2 in the inspired air are tolerated, with an increase of respiratory rate as the main effect. Higher levels cause dyspnoea (shortness of breath), headaches, which are thought to result from cerebral vasodilation caused by the elevated $paCO_2$, restlessness, faintness, dulling of consciousness, greatly elevated alveolar ventilation, muscular rigidity and tremors occur at inspired CO_2 concentrations greater than 15%. At 20% to 30% inspired CO_2, generalized convulsions can be produced [92].

In several lung diseases there is inadequate gas exchange which results in accumulation of CO_2 in the body to levels above 50 mmHg. The effects of high pCO_2 on the lung epithelium and how hypercapnia contributes to disease progression have not been fully elucidated. A significant number of patients with chronic obstructive pulmonary disease have elevated pCO_2 levels, which is associated with worse outcome [93–96]. In patients with pulmonary oedema and acute respiratory distress syndrome, there is impairment in pulmonary gas exchange and poor patient outcome unless corrective measures are taken to resolve the oedema and re-establish normal epithelial barrier function [97–100]. During respiratory failure an important measure is the use of mechanical ventilation. However, ventilation at levels that restore pulmonary CO_2 levels to normal frequently results in 'ventilator-induced lung

injury'. There is significant evidence that ventilator-induced lung injury can be reduced through the use of 'permissive hypercapnia' regimes in which less aggressive ventilation is used and CO_2 levels are allowed to rise as high as 100 mmHg [101–106]. $paCO_2$ levels as high as 250 mmHg have been reported in patients with uncontrolled asthma [107, 108]. The use of 'permissive hypercapnia' has been supported by results from experimental models where hypercapnic acidosis has been reported to be protective against ischemia reperfusion and ventilator-induced lung injury [104, 109–111]. However, more recent studies have suggested that hypercapnia may have deleterious effects on the lungs such as impaired alveolar fluid absorbance [112–116], which questions the clinical use of the 'permissive hypercapnia' [112–117].

Hypercapnia leads to impaired lung oedema clearance, a major function of the alveolar epithelium [59]. The changes triggered by the alveolar epithelium appear to be independent of pH, as metabolic acidosis does not have an effect on fluid clearance, whereas hypercapnia with normal pH levels lead to decreased fluid clearance. The deleterious effects of hypercapnia on the alveolar epithelia are not due to hypoxia, nor are they mediated by CAs [118]. Effects of hypercapnia on alveolar epithelia have been observed for up to 7 days in rats and at least in the short term, are reversible upon normalization of CO_2 levels [59]. The effects of hypercapnia on alveolar fluid clearance are also reversed by the treatment of rats with β-adrenergic agonists [60].

Mechanical ventilation that alters $paCO_2$ and paO_2, may also affect vascular dynamics *via* activation or inactivation of vasoactive factors such as nitric oxide, angiotensin II, endothelin and bradykinin [119]. Hypercapnia is inversely correlated with renal blood flow (RBF) and causes renal constriction. The direct mechanisms include activation of the sympathetic nervous system through release of norepinephrine. The increased sympathetic activity reduces RBF and glomerular filtration rate and contributes to a non-osmotic release of vasopressin. The indirect mechanism is a decrease in systemic vascular resistance due to systemic vasodilatation. The decrease leads to further release of norepinephrine and stimulation of the renin–angiotensin–aldosterone system, causing decreased RBF. These hypercapnic effects occur independently of paO_2 and determine the renovascular response to changes in arterial blood gas parameters [119].

Physiological effects on *D. melanogaster*

Drosophila and other insects are routinely anesthetized by using elevated CO_2 levels. Exposure to 100% CO_2 for a few seconds is sufficient to render adult flies and larvae immobile and easy to handle. In this paralysed state, the animals are non-responsive to mechanical sensory stimuli [120]. Therefore, the effects of CO_2 on fly physiology have primarily been considered in the context of potential side effects resulting from its use as an anaesthetic, usually an acute pulse of 100% CO_2. Fewer studies have explored the physiological effects of chronic exposure (hours to weeks) to non-anaesthetic ($<$30%) levels of CO_2.

The physiological consequences of CO_2 anaesthesia are unlikely to be encountered naturally and therefore are not discussed in much detail here. However, anaesthetic levels of CO_2 increase copulation latency or female sexual receptivity, even after a 20 hr recovery time in normal air [121]. Placed in 100% CO_2, the body wall movements of *D. melanogaster* larva initially cease within 40 sec., but are followed by rapid contractions and bending, and then elongation and unresponsiveness [120]. In contrast, exposure of larvae to 100% N_2 does not cause these effects even after 10 min., indicating that hypoxia is not the cause of the behaviour. A total of 100% CO_2 also slows larval heart rate, independently of haemolymph pH. Further, these effects are observed even in larvae whose central nerve system had been removed. The sensitivity of the skeletal neuromuscular junction to glutamate is also reduced by extreme CO_2 exposure, which may explain the loss in motor ability during CO_2 anaesthesia. Pupae exposed to anaesthetic levels of CO_2 develop slower and have a lower dry weight at eclosion [122]. Another study showed that CO_2 anaesthesia affects survival and fecundity only when used on very young male flies (0 to 3 hrs old) [123]; however, post-anaesthetic activity levels of all flies are increased [124]. Interestingly, CO_2 anaesthesia is toxic within 30 sec. to flies infected with the sigma virus [125]. CO_2 also increases recovery time from a coma induced by cold temperatures, presumably due to CO_2 affecting the neuromuscular junction, because chill coma recovery is thought to be influenced by muscular excitability [126].

What are the physiological effects on *D. melanogaster* of prolonged exposure to non-anaesthetic levels of CO_2? A recent study [77] shows that *D. melanogaster* adult females lay fewer eggs in 13% CO_2 and almost no eggs at 20% CO_2. Unlike *C. elegans* (see below), CO_2 up to 13% does not affect lifespan of fruit flies, suggesting that fly health is not globally affected. Embryonic *D. melanogaster* development is severely disrupted in 20% CO_2, and significantly slowed down in 13% CO_2, with some embryos showing morphological abnormalities. Interestingly, when adult flies are infected with bacteria and exposed to CO_2 levels as low as 7%, they suffer increased mortality compared to their counterparts cultured in air. This mortality is caused by a defect in host resistance, as CO_2-exposed flies harboured significantly more bacteria compared to those in air, independent of any effects of CO_2 on bacterial growth [77].

Physiological effects on *C. elegans*

In response to CO_2 levels of 9% and above, *C. elegans* show specific phenotypes that are independent of pH changes in the growth media [18]. These phenotypes include reduced number of laid eggs, which developed with normal morphology but at a reduced rate, and a reduced pharyngeal pumping rate, which returned to normal when the animals were returned to normocapnia. In addition, chronic exposure to CO_2 caused a reduction in motility, probably due to the deterioration of striated muscle (Fig. 1). The effect of chronic exposure to elevated CO_2 levels on muscle cells are especially intriguing, because patients with

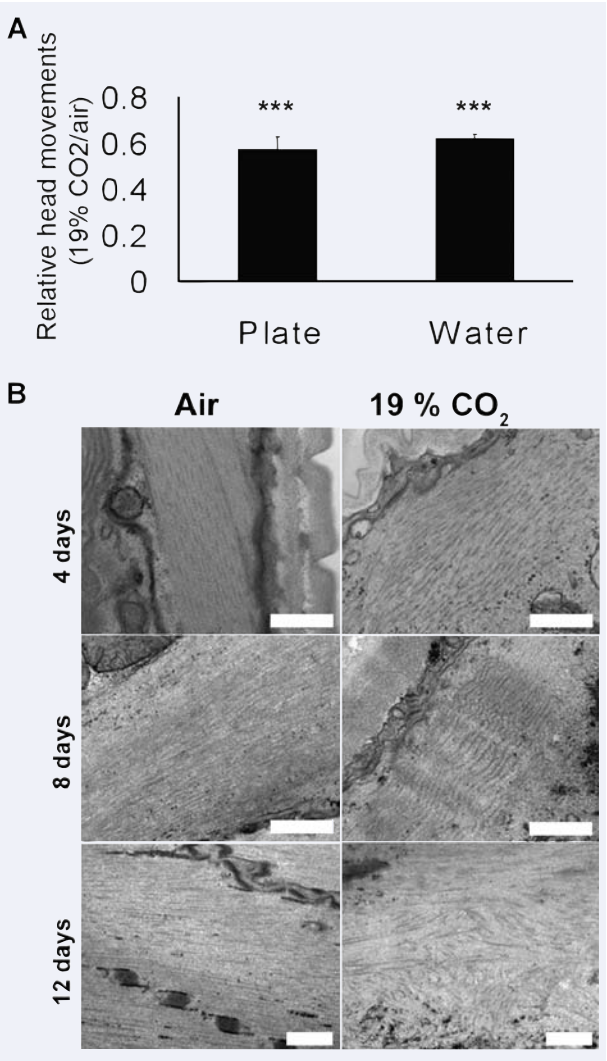

Fig. 1 Growth in air containing 19% CO_2 reduces motility and affects muscle morphology in *C. elegans*. (**A**) The average number of head movements of wild-type (N2) animals at the L4 larval stage grown in air or in air containing 19% CO_2 on agar plates or in a water drop. All measurements were performed after the animals were removed from the CO_2 chamber. The number of head movements/minute was divided by the average number of head movement of animals grown in air. (**B**) Thin-section electron micrographs demonstrating the gradual deterioration of body muscles in animals grown for 4, 8 or 12 days in air containing 19% CO_2 at 20°C. Muscle morphology was normal in animals grown in air. The muscle of animals grown in air containing 19% CO_2 had deteriorated already at day 4 and muscle filaments were further disorganized at days 8 and 12. Scale bars = 500 nm. The data were taken from Ref. [18].

chronic obstructive pulmonary disease show reduction in muscle mass and increased ubiquitination and proteolysis [127, 128]. The *C. elegans* phenotypes were more severe with increasing levels of CO_2 (up to 19%), and were accompanied with specific and dynamic changes in transcription (see section 'Molecular responses in *C. elegans*').

Importantly, there was also a significant increase in lifespan [18]. This lifespan increase is probably independent of the IGF-1 pathway, because both the short-lived *daf-16*(*mu86*) and the long-lived *daf-2*(*e1370*) mutants still show significant CO_2-dependent extension in lifespan. The lifespan extension was also independent of mitochondria-mediated aging, egg laying, diet restriction or diet deprivation, because *clk-1*(*e2519*), *eat-2*(*ad1116*) and *glp-1* (*or178*) mutant animals all showed extension in lifespan [18] (K.S. and Y.G., unpublished observations).

Molecular responses to elevated CO_2 levels

Molecular responses in mammalian neuronal cells

It is not clear yet whether mammalian chemosensitive neurons sense cellular pH, a pH gradient across the membrane, CO_2 or HCO_3 Therefore, it is not surprising that the receptors that directly sense CO_2 levels have not yet been defined. HCO_3^- (but not CO_2 or change in pH) causes direct activation of a 48 kD sAC protein [129]. In mammalian cells, soluble sAC is targeted to well-defined intracellular compartments including mitochondria, centrioles, mitotic spindles, mid-bodies and nuclei, where it responds to intrinsic cellular signals and can regulate cell metabolism [130, 131]. Under conditions of elevated CO_2 levels, there is an increase in HCO_3^- levels leading to increase in sAC and cyclic AMP (cAMP) levels [130] (Fig. 2). The cAMP activates PKA, which in turn leads to opening of L-type Ca^{2+} channels and influx of Ca^{2+} into cells. [22]. Indeed, in the glomus cells of the carotid nucleus, increased CO_2 levels cause the activation of L-type Ca^{2+} channels and an elevated Ca^{2+} influx [132–134]. Experiments in isolated glomus cells showed that the activation of Ca^{2+} channels and the elevated Ca^{2+} influx are independent of changes in pH [133] (Fig. 2).

Molecular response to elevated levels of CO_2 in mammalian non-neuronal cells

Lung cells

Although the sensing of CO_2 and the mechanisms by which high CO_2 levels lead to alveolar dysfunction (*i.e.* impaired alveolar fluid clearance) have not been fully elucidated, significant progress has been made in the understanding of some of the signalling pathways. Acute hypercapnia leads to impaired function of the alveolar epithelial Na,K-ATPase due to its endocytosis [59, 60]. The signalling pathway leading to Na,K-ATPase endocytosis starts with an increase in cytosolic Ca^{2+}, which leads to activation of the Ca^{2+}-calmodulin dependent protein kinase β (Fig. 3). This kinase phosphorylates the AMP-activated protein kinase at Thr172 in the activation loop of its α subunit, which in turn activates the

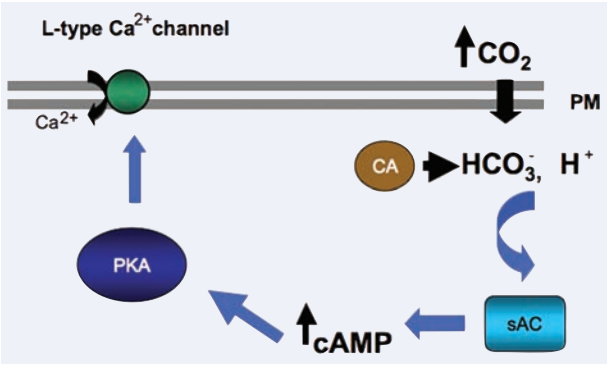

Fig. 2 Schematic model of the pathway leading to hypercapnia-induced activation of L-type Ca^{2+} channels in carotid neurons. Elevated CO_2 levels are converted by CA to protons and HCO_3^-. The carbonate directly activates the expression of sAC, which converts ATP to cyclic AMP (cAMP). The cAMP activates PKA, which is believed to activate the L-type Ca^{2+} channels leading to Ca^{2+} entry into cells. This model is based on a model proposed in [22].

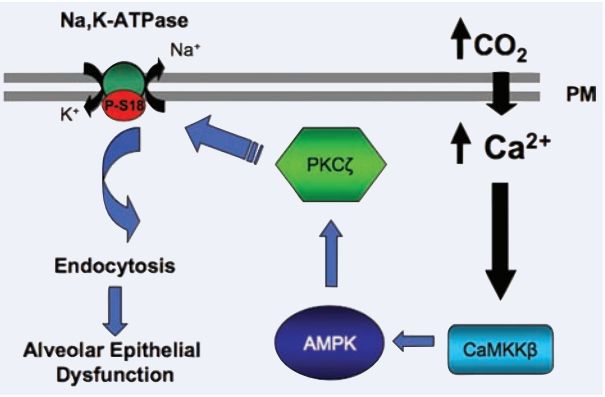

Fig. 3 Schematic model of the pathway leading to hypercapnia-induced Na,K-ATPase endocytosis in alveolar epithelial cells. Elevated CO_2 levels initiate an intracellular Ca^{2+}-dependent signalling pathway that involves the activation of CaMKK-β, AMPK and PKC-ζ, which in turns phosphorylates the Na,K-ATPase α_1-subunit at Ser18, triggering its endocytosis and thus impairing AFR, an essential function of the alveolar epithelium.

atypical PKC-ζ. Phosphorylation of the Na,K-ATPase α_1-subunit at Ser 18 by PKC-ζ promotes its endocytosis resulting in decreased Na,K-ATPase activity in alveolar epithelial cells and accompanying pulmonary oedema [59, 60] (Fig. 3).

Kidney cells

A major task of the kidney is to secrete H^+ into the urine, and thus hypercapnia rapidly stimulates renal H^+ secretion [135, 136]. The renal proximal tubule reabsorbs ~80% of the HCO_3^- filtered by the glomerulus by secreting H^+ into the proximal tubule lumen and using this H^+ to titrate luminal HCO_3^- to CO_2 and H_2O. After entering the cell across the apical membrane, the CO_2 and H_2O recombine to produce H^+ and HCO_3^-. Extrusion of H^+ into the lumen across the apical membrane is carried out by Na-H exchangers [137] and H^+ pumps [138] and HCO_3^- is transported across the basolateral membrane *via* the electrogenic Na^+-HCO_3^- co-transporter NBCe1-A [139]. Although there is not much information on the molecular mechanisms involved in renal H^+ secretion induced by high levels of CO_2, it has been described that respiratory acidosis stimulates activity of renal H^+ ATPases [136], probably by stimulating their recruitment towards the plasma membrane [140]. Other work has indicated that an as yet unidentified receptor tyrosine kinase is required for responses of rabbit proximal tubule cells to changes in CO_2 levels [58].

Molecular responses in *D. melanogaster*

Olfactory responses

A critical clue to the molecular mechanisms mediating CO_2 responses came from observations that there is a single population of neurons expressing the candidate gustatory receptor Gr21a, which is required for sensing gaseous CO_2 in both adult flies and larvae [74, 76]. Subsequent genetic and molecular experiments showed that the Gr21a receptor is not merely a marker of CO_2-responsive neurons, but is required for sensing CO_2 [67, 68]. The ab1C neurons also express another gustatory receptor, Gr63a. Ectopic expression of both the receptors together, but not either alone, conferred CO_2 responsiveness to neurons that were previously unresponsive to CO_2. The response is highly specific and dose dependent, with ab1C neurons starting to fire immediately upon increasing CO_2 concentration above ambient, while in the ectopic system 3% CO_2 was readily detected. Flies lacking Gr63a fail to avoid CO_2 in a T-maze assay, similar to flies in which the Gr21a-expressing neurons had been silenced. Thus, a Gr21a/Gr63a heterodimer is thought to be necessary and sufficient for sensing CO_2 [67, 68]. Gr63a and Gr21a are highly conserved across *Drosophilid* genomes, and paralogs of Gr21a are present in mosquitoes, the silk moth and red flour beetle [141]. However, although ancient, the Gr21a and Gr63a gene lineages are absent from all other arthropods whose genomes have been sequenced, even though some of these species are known to sense CO_2.

It is not yet known how Gr21a and Gr63a detect CO_2, and what the role of each receptor is. For example, it is unclear if one acts as a signalling or localization cofactor or chaperone, while the other binds ligand or if both receptors are required for both functions. Likewise, it is not yet known if the signalling ligand is CO_2 itself, bicarbonate or perhaps CO_2 bound to some other as yet unknown factor. Neither study in which the CO_2 receptors were identified examined the pH-sensitivity of the receptors. In addition, at present nothing is known about the signal transduction cascade downstream of these receptors.

Possible molecular functions of the CO_2-responsive gustatory receptors (Grs) Gr21a and Gr63a can be derived from advances in understanding the function of the fly olfactory receptors (Ors). Grs

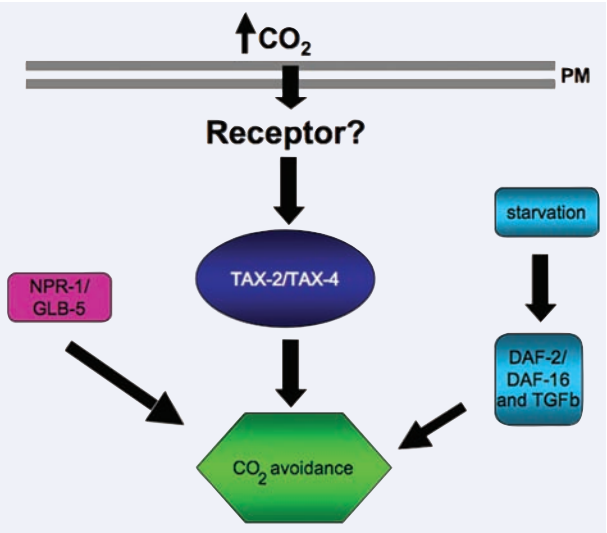

Fig. 4 Schematic model of the pathway leading to CO_2 avoidance in *C. elegans* CO_2 avoidance is mediated by the cGMP signalling pathway molecules TAX-2 and TAX-4 expression in the BAG neurons. The avoidance behaviour is also modulated by the neuropeptide Y receptor NPR-1, by the neuronal globin domain protein GLB-5, and by the insulin and TGF-β starvation pathways.

and Ors are distantly related [142, 143] and together define a novel superfamily of insect chemoreceptors [144]. Two recent papers showed that in a heterologous *in vitro* system, *Drosophila* Ors function as ligand-gated ion channels that respond to odorants [145, 146]. Although Grs and Ors, like GPCRs, are 7-transmembrane spanning proteins, it is not obvious that they transduce signals *via* G proteins as they have no homology to GPCRs and their membrane topology is inverted compared to GPCRs [147]. Nonetheless, G-protein signalling may still be involved in fly olfaction as Wicher *et al.* [145] were able to demonstrate that odorants stimulate a cAMP second messenger system, and propose that cyclic nucleotides gate the Or ion channel. Production of cyclic nucleotides was prevented using a G protein inhibitor, suggesting that odour detection might activate an adenylyl or guanylyl cyclase. However, Sato *et al.* [146] did not find evidence for roles of cAMP olfaction, indicating that additional work is needed to definitively understand the olfactory signalling pathway.

Could cyclic nucleotide-gated ion channels represent a general conserved mechanism for CO_2 sensing? Such a possibility is supported by evidence that in *C. elegans*, *tax-2* and *tax-4* are subunits of an ion channel gated by cyclic GMP required for the avoidance behaviour to CO_2 (see below). Further, a recent study show that bicarbonate can activate guanylyl cyclases in CO_2-responsive neurons in mice [148], and the responses to CO_2 are known to be dependent on the opening of cGMP-sensitive cyclic nucleotide-gated channels [149]. Also, as discussed above, the cyclic nucleotide cAMP appears to mediate effects of elevated CO_2 levels in fungi [19], and adenylyl cyclases can be activated by bicarbonate in mammalian sperm [129, 150] and by molecular CO_2 in cyanobacteria [151].

Gustatory responses

The molecular basis for detection of carbonation is not presently known. The E409 neurons that respond to CO_2 in solution do not express Gr21a or Gr63a [67, 75], and Gr63a mutants respond to carbonation equally well to wild-type flies [69], indicating that the molecular mechanisms of sensing gaseous and soluble CO_2 are distinct.

Non-neuronal responses

Separate from neuronal responses to CO_2, non-neuronal *Drosophila* cells also respond to CO_2. Microarray studies on adult flies show that elevated but non-anaesthetic CO_2 levels (13%) induce a specific transcriptional signature in adult *D. melanogaster*, which is different from that induced by other stresses such as heat shock, hypoxia and oxidative stress [77]. Notably, genes required for egg production are strongly down-regulated by a 24 hr exposure to 13% CO_2 [77], which account for the reductions in egg laying by both wild-type and Gr63a null flies in 13% CO_2. Elevated CO_2 levels also suppress some innate immune effector genes in adult flies, which may account for the decrease in host resistance and increased mortality observed during bacterial infection [77]. Suppression of antimicrobial peptides was also observed in an immune-responsive S2* cell line, and was independent of extracellular pH. Critically, the deleterious *in vivo* effects of CO_2 on fecundity and bacterial resistance are still observed in Gr63a mutant flies. Taken together, these results suggest that the fly possesses as yet unidentified CO_2 detection mechanisms, which are non-neuronal and can be cell autonomous. These mechanisms may be conserved, because CO_2 may also suppress innate immune responses in mammalian systems.

Molecular responses in *C. elegans*

The TAX-2 and TAX-4 subunits of a cGMP gated ion channel are essential for the CO_2 avoidance behaviour [80], because *tax-2* and *tax-4* mutants do not avoid CO_2 [78, 79]. Expression of TAX-4 in the BAG neurons alone was sufficient to recover the CO_2 avoidance defect of *tax-4* mutants, which implicates the involvement of these neurons in CO_2 avoidance (Fig. 4). Importantly, *C. elegans* do not avoid CO_2 in all conditions; starved animals do not avoid CO_2. This implicates metabolic regulatory pathways in modulating response to CO_2, and indeed mutants with reduced *daf-2* signalling, which mimics starvation condition; do not avoid CO_2 [78, 79] (Fig. 4). Genetically interfering with the feeding behaviour *via* the IGF-1 or the TGF-β pathways can change the way N2 animals respond to CO_2, N2 animals with null mutation in the neuropeptide Y receptor *npr-1* change their feeding behaviour from solitary to social and accordingly change the way they respond to CO_2 from CO_2 sensitive to CO_2 insensitive [80]. The *npr-1* signalling and the neuronal globin domain protein, GLB-5 also affect the response to

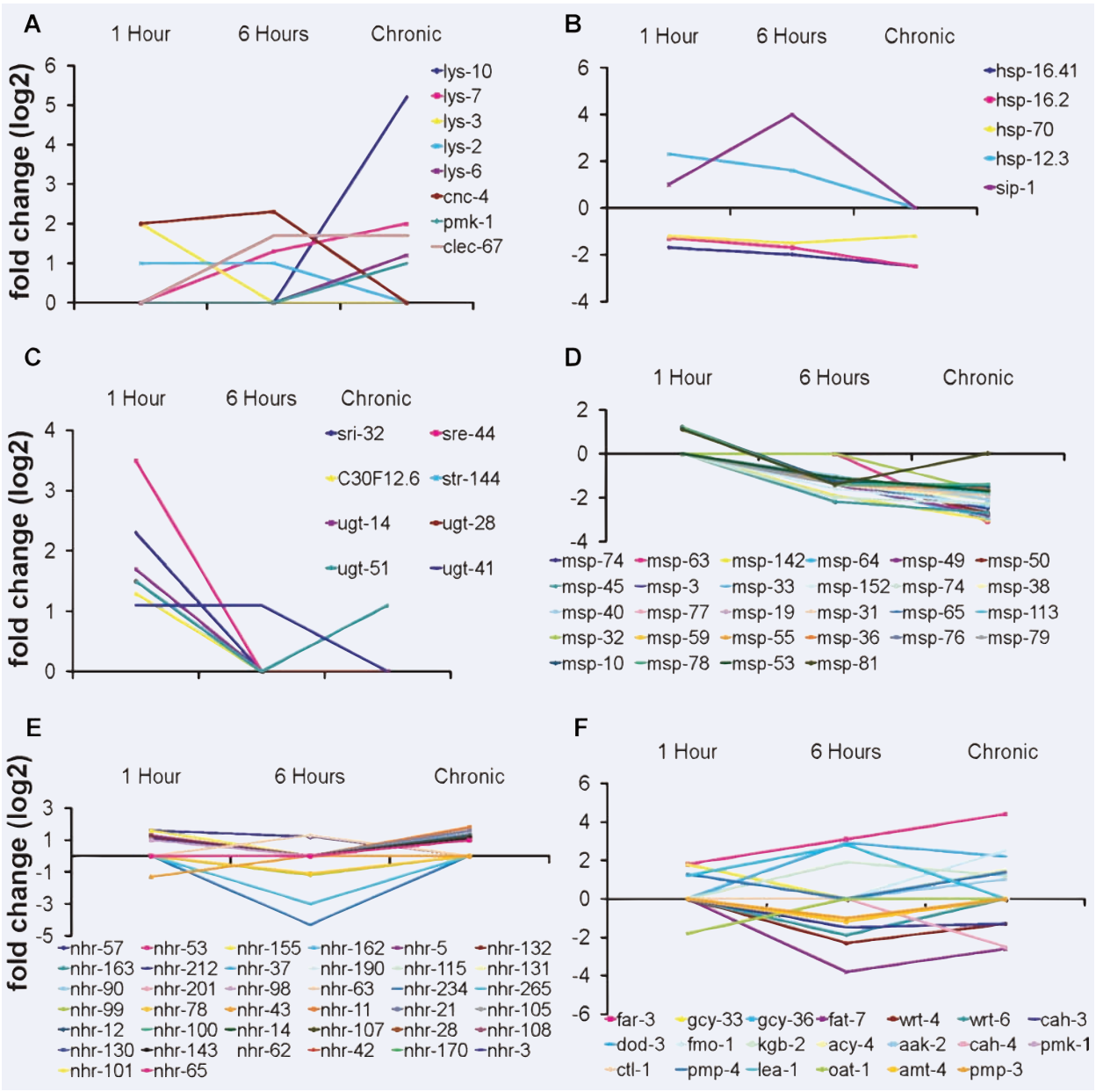

Fig. 5 Hypercapnia induces change in gene expression in *C. elegans*. Fold change in $\log_2$ scale of gene expression during 1, 6 or 72 hrs exposure to air containing 19% CO_2 of innate immunity (**A**), heat shock (**B**), 7-transmembrane domain (**C**), major sperm proteins (**D**), nuclear hormone receptor (**E**) and several other genes of interest. The data were taken from [18].

O_2, which implicates a potential relationship between the CO_2 and O_2 responses; however, soluble guanylyl cyclases (sGC) which serve as oxygen sensors in *C. elegans* do not seem to be involved at the avoidance response from CO_2 because animals mutants in sGC genes avoid CO_2 normally [80].

The development, fertility, motility and aging phenotypes detected in *C. elegans* grown in >9% CO_2 levels conditions are accompanied with changes in the expression of specific genes (Fig. 5). These changes are dynamic and a large number of genes are already changed more than 2-fold after 1-hr exposure to 19% CO_2. After a 6-hr exposure to hypercapnia, most of the genes affected after a 1-hr exposure to hypercapnia had returned to their baseline-expression levels, but expression of many other genes not affect by the 1-hr exposure were now changed.

Following a 72-hr exposure to 19% CO_2, over 6% of *C. elegans* genes were either up-regulated or down-regulated at least 2-fold. Many of the genes that were up-regulated following 1-hr exposure to hypercapnia are probably involved in coordinating the initial response of the animal to hypercapnia, including several 7-transmembrane domain and hormone receptors, sGC and ubiquitin ligase genes (Fig. 5).

In summary, we review here the effects of hypercapnia on mammals, *Drosophila* and *C. elegans*. High CO_2 levels appear to be sensed by cells independent of pH or O_2 *via* specific signalling pathways, which results in distinct effects (phenotypes). Studies in mice, *Drosophila* and *C. elegans* may provide valuable insights into the effects of hypercapnia on human health.

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References

1. **Missner A, Kügler P, Saparov SM, *et al.*** Carbon dioxide transport through membranes. *J Biol Chem*. 2008; 37: 25340–7.
2. **Grime JM, Edwards MA, Rudd NC, *et al.*** Quantitative visualization of passive transport across bilayer lipid membranes. *Proc Natl Acad Sci USA*. 2008; 105: 14277–87.
3. **Waisbren SJ, Geibel JP, Modlin IM, *et al.*** Unusual permeability properties of gastric gland cells. *Nature*. 1994; 368: 332–5.
4. **Forster RE, Gros G, Lin L, *et al.*** The effect of 4,4'-diisothiocyanato-stilbene-2,2'-disulfonate on CO2 permeability of the red blood cell membrane. *Proc Natl Acad Sci USA*. 1998; 95: 15815–20.
5. **Uehlein N, Lovisolo C, Siefritz F, *et al.*** The tobacco aquaporin NtAQP1 is a membrane CO2 pore with physiological functions. *Nature*. 2003; 425: 734–7.
6. **Uehlein N, Otto B, Hanson DT, *et al.*** Function of Nicotiana tabacum aquaporins as chloroplast gas pores challenges the concept of membrane CO2 permeability. *Plant Cell*. 2008; 20: 648–57.
7. **Marini AM, Urrestarazu A, Beauwens R, *et al.*** The Rh (rhesus) blood group polypeptides are related to NH4+ transporters. *Trends Biochem Sci*. 1997; 22: 460–1.
8. **Soupene E, Inwood W, Kustu S.** Lack of the Rhesus protein Rh1 impairs growth of the green alga Chlamydomonas reinhardtii at high CO2. *Proc Natl Acad Sci USA*. 2004; 101: 7787–92.
9. **Soupene E, King N, Feild E, *et al.*** Rhesus expression in a green alga is regulated by CO(2). *Proc Natl Acad Sci USA*. 2002; 99: 7769–73.
10. **Endeward V, Musa-Aziz R, Cooper GJ, *et al.*** Evidence that aquaporin 1 is a major pathway for CO2 transport across the human erythrocyte membrane. *FASEB J*. 2006; 20: 1974–81.
11. **Bruce LJ, Beckmann R, Ribeiro ML, *et al.*** A band 3-based macrocomplex of integral and peripheral proteins in the RBC membrane. *Blood*. 2003; 101: 4180–8.
12. **Bruce LJ.** Red cell membrane transport abnormalities. *Curr Opin Hematol*. 2008; 15: 184–90.
13. **Endeward V, Cartron JP, Ripoche P, *et al.*** RhAG protein of the Rhesus complex is a CO2 channel in the human red cell membrane. *FASEB J*. 2008; 22: 64–73.
14. **Tanner MJ.** Band 3 anion exchanger and its involvement in erythrocyte and kidney disorders. *Curr Opin Hematol*. 2002; 9: 133–9.
15. **Herrera M, Hong NJ, Garvin JL.** Aquaporin-1 transports NO across cell membranes. *Hypertension*. 2006; 48: 157–64.
16. **Musa-Aziz R, Chen LM, Pelletier MF, *et al.*** Relative CO2/NH3 selectivities of AQP1, AQP4, AQP5, AmtB, and RhAG. *Proc Natl Acad Sci USA*. 2009; 106: 5406–11.
17. **Ji Q, Hashmi S, Liu Z, *et al.*** CeRh1 (rhr-1) is a dominant Rhesus gene essential for embryonic development and hypodermal function in Caenorhabditis elegans. *Proc Natl Acad Sci USA*. 2006; 103: 5881–6.
18. **Sharabi K, Hurwitz A, Simon AJ, *et al.*** Elevated CO2 levels affects development, motility, fertility and extends lifespan in Caenorhabditis elegans. *Proc Natl Acad Sci USA*. 2009; 106: 4024–9.
19. **Bahn YS, M¸hlschlegel FA.** CO2 sensing in fungi and beyond. *Curr Opin Microbiol*. 2006; 9: 572–8.
20. **Klengel T, Liang WJ, Chaloupka J, *et al.*** Fungal adenylyl cyclase integrates CO2 sensing with cAMP signaling and virulenc. *Curr Biol*. 2005; 15: 2021–6.
21. **Forster HV, Martino P, Hodges M, *et al.*** The carotid chemoreceptors are a major determinant of ventilatory CO2 sensitivity and of PaCO2 during eupneic breathing. *Adv Exp Med Biol*. 2008; 605: 322–6.
22. **Putnam RW, Filosa JA, Ritucci NA.** Cellular mechanisms involved in CO2 and acid signaling in chemosensitive neurons. *Am J Physiol Cell Physiol*. 2004; 287: C1493–526.
23. **Peers C, Buckler KJ.** Transduction of chemostimuli by the type I carotid body cell. *J Membr Biol*. 1995; 144: 1–9.
24. **Daristotle L, Berssenbrugge AD, Engwall MJ, *et al.*** The effects of carotid body hypocapnia on ventilation in goats. *Respir Physiol 79*. 1990: 123–35.
25. **Lahiri S, DeLaney RG.** Relationship between carotid chemoreceptor activity and ventilation in the cat. *Respir Physiol*. 1975; 24: 267–86.
26. **Smith CA, Rodman JR, Chenuel BJ, *et al.*** Response time and sensitivity of the ventilatory response to CO2 in unanesthetized intact dogs: central vs. peripheral chemoreceptors. *J Appl Physiol*. 2006; 100: 13–9.
27. **Hodges MR, Opansky C, Qian B, *et al.*** Carotid body denervation alters ventilatory responses to ibotenic acid injections or focal acidosis in the medullary raphe. *J Appl Physiol*. 2005; 98: 1234–42.
28. **Bisgard GE, Forster HV, Orr JA, *et al.*** Hypoventilation in ponies after carotid body denervation. *J Appl Physiol*. 1976; 40: 184–90.
29. **Lowry TF, Forster HV, Pan LG, *et al.*** Effects on breathing of carotid body denervation in neonatal piglets. *J Appl Physiol*. 1999; 87: 2128–35.
30. **Rodman JR, Curran AK, Henderson KS, *et al.*** Carotid body denervation in dogs: eupnea and the ventilatory response to hyperoxic hypercapnia. *J Appl Physiol*. 2001; 91: 328–35.

31. **Pan LG, Forster HV, Martino P, *et al.*** Important role of carotid afferents in control of breathing. *J Appl Physiol*. 1998; 85: 1299–306.
32. **Iturriaga R, Mokashi A, Lahiri S.** Dynamics of carotid body responses *in vitro* in the presence of CO2-HCO3-: role of carbonic anhydrase. *J Appl Physiol*. 1993; 75: 1587–94.
33. **Fidone SJ, Gonzalez C.** Handbook of Physiology. The Respiratory System. Control of Breathing. Bethesda: American Physiological Association; 1986.
34. **Hanson MA, Nye PCG, Torrance RW.** The exodus of an extracellular bicarbonate theory of chemoreception and the genesis of an intracellular one. Leicester: Leicester University Press; 1971.
35. **Nattie G, Li A.** Multiple central chemoreceptor sites: cell types and function in vivo. *Adv Exp Med Biol*. 2008; 605: 343–7.
36. **Ainslie PN, Duffin J.** Integration of cerebrovascular CO2 reactivity and chemoreflex control of breathing: mechanisms of regulation, measurement, and interpretation. *Am J Physiol Regul Integr Comp Physiol*. 2009; 296: R1473–95.
37. **Carpenter DO, Hubbard JH, Humphrey DR, *et al.*** Carbon dioxide effects on nerve cell function. New York: Springer; 1974.
38. **Huang RQ, Erlichman JS, Dean JB.** Cell-cell coupling between CO2-excited neurons in the dorsal medulla oblongata. *Neuroscience*. 1997; 80: 41–57.
39. **Wellner-Kienitz MC, Shams H.** CO2-sensitive neurons in organotypic cultures of the fetal rat medulla. *Respir Physiol*. 1998; 111: 137–51.
40. **Erlichman JS, Li A, Nattie EE.** Ventilatory effects of glial dysfunction in a rat brain stem chemoreceptor region. *J Appl Physiol*. 1998; 85: 1599–64.
41. **Mulkey DK, Henderson RA, Putnam RW, *et al.*** Hyperbaric oxygen and chemical oxidants stimulate CO2/H+-sensitive neurons in rat brain stem slices. *J Appl Physiol*. 2003; 95: 910–21.
42. **Ritucci NA, Dean JB, Putnam RW.** Intracellular pH response to hypercapnia in neurons from chemosensitive areas of the medulla. *Am J Physiol*. 1997; 273: R433–41.
43. **Borison HL, Hurst JH, McCarthy LE, *et al.*** Arterial hydrogen ion versus CO2 on depth and rate of breathing in decerebrate cats. *Respir Physiol*. 1997; 30: 311–25.
44. **Wang W, Pizzonia JH, Richerson GB.** Chemosensitivity of rat medullary raphe neurones in primary tissue culture. *J Physiol*. 1998; 511: 433–50.
45. **Fukuda Y.** Difference between actions of high PCO2 and low [HCO-3] on neurons in the rat medullary chemosensitive areas *in vitro*. *Pflugers Arch*. 1983; 398: 324–30.
46. **Gupte SA, Wolin MS.** Oxidant and redox signaling in vascular oxygen sensing: implications for systemic and pulmonary hypertension. *Antioxid Redox Signal*. 2008; 10: 1137–352.
47. **Bryan NS, Bian K, Murad F.** Discovery of the nitric oxide signaling pathway and targets for drug development. *Front Biosci*. 2009; 14: 1–18.
48. **Nedvetsky PI, Kleinschnitz C, Schmidt HH.** Regional distribution of protein and activity of the nitric oxide receptor, soluble guanylyl cyclase, in rat brain suggests multiple mechanisms of regulation. *Brain Res*. 2002; 950: 148–54.
49. **Bilban M, Haschemi A, Wegiel B, *et al.*** Heme oxygenase and carbon monoxide initiate homeostatic signaling. *J Mol Med*. 2008; 86: 267–79.
50. **Chen ML, Keens TG.** Congenital central hypoventilation syndrome: not just another rare disorder. *Paediatr Respir Rev*. 2004; 5: 182–9.
51. **Gozal D.** Congenital central hypoventilation syndrome: an update. *Pediatr Pulmonol*. 1998; 26: 273–82.
52. **Guyenet PG.** The 2008 Carl Ludwig Lecture: retrotrapezoid nucleus, CO2 homeostasis, and breathing automaticity. *J Appl Physiol*. 2008; 105; 404–16.
53. **Amiel J, Laudier B, Attie-Bitach T, *et al.*** Polyalanine expansion and frameshift mutations of the paired-like homeobox gene PHOX2B in congenital central hypoventilation syndrome. *Nat Genet*. 2003; 33: 459–60.
54. **Weese-Mayer DE, Berry-Kravis EM, Zhou L, *et al.*** Idiopathic congenital central hypoventilation syndrome: analysis of genes pertinent to early autonomic nervous system embryologic development and identification of mutations in PHOX2b. *Am J Med Genet*. 2003; 123A: 267–78.
55. **Dubreuil V, Ramanantsoa N, Trochet D, *et al.*** A human mutation in Phox2b causes lack of CO_2 chemosensitivity, fatal central apnea, and specific loss of parafacial neurons. *Proc Natl Acad Sci USA*. 2008; 105: 1067–72.
56. **Takakura AC, Moreira TS, Stornetta RL, *et al.*** Selective lesion of retrotrapezoid Phox2b-expressing neurons raises the apnoeic threshold in rats. *J Physiol*. 2008; 586: 2975–91.
57. **Zhou Y, Zhao J, Bouyer P, *et al.*** Evidence from renal proximal tubules that HCO3- and solute reabsorption are acutely regulated not by pH but by basolateral HCO3- and CO2. *Proc Natl Acad Sci USA*. 2005; 102: 3875–80.
58. **Zhou Y, Bouyer P, Boron WF.** Role of a tyrosine kinase in the CO_2-induced stimulation of HCO_3-reabsorption by rabbit S2 proximal tubules. *Am J Physiol Renal Physiol*. 2006; 291: F358–67.
59. **Briva A, Lecuona E, Welch L, *et al.*** High CO2 levels impair alveolar epithelial function independently of pH. *PLos ONE*. 2007; 2: e1238.
60. **Vadasz I, Dada LA, Briva A, *et al.*** AMP-activated protein kinase regulates CO2-induced alveolar epithelial dysfunction in rats and human cells by promoting Na,K-ATPase endocytosis. *J Clin Invest*. 2008; 118: 752–62.
61. **Takeshita K, Suzuki Y, Nishio K, *et al.*** Hypercapnic acidosis attenuates endotoxin-induced nuclear factor-[kappa]B activation. *Am J Respir Cell Mol Biol*. 2003; 29: 124–32.
62. **West MA, Hackam DJ, Baker J, *et al.*** Mechanism of decreased *in vitro* murine macrophage cytokine release after exposure to carbon dioxide: relevance to laparoscopic surgery. *Ann Surg*. 1997; 226: 179–90.
63. **Lang CJ, Dong P, Hosszu EK, *et al.*** Effect of CO2 on LPS-induced cytokine responses in rat alveolar macrophages. *Am J Physiol Lung Cell Mol Physiol*. 2005; 289: L96–103.
64. **Stange G, Stowe S.** Carbon-dioxide sensing structures in terrestrial arthropods. *Microsc Res Tech*. 1999; 47: 416–27.
65. **Vosshall LB, Stocker RF.** Molecular architecture of smell and taste in *Drosophila*. *Annu Rev Neurosci*. 2007; 30: 505–33.
66. **Gillies MT.** The role of carbon dioxide in host-finding in mosquitoes. *Bull Entomol Res*. 1980; 70: 525–32.
67. **Jones WD, Cayirlioglu P, Kadow IG, *et al.*** Two chemosensory receptors together mediate carbon dioxide detection in *Drosophila*. *Nature*. 2007; 445: 86–90.
68. **Kwon JY, Dahanukar A, Weiss LA, *et al.*** The molecular basis of CO2 reception in *Drosophila*. *Proc Natl Acad Sci USA*. 2007; 104: 3574–8.
69. **Fischler W, Kong P, Marella S, *et al.*** The detection of carbonation by the *Drosophila* gustatory system. *Nature*. 2007; 448: 1054–7.
70. **Dunipace L, Meister S, McNealy C, *et al.*** Spatially restricted expression of candidate taste receptors in the *Drosophila* gustatory system. *Curr Biol*. 2001; 11: 822–35.

71. **de Bruyne M, Warr CG.** Molecular and cellular organization of insect chemosensory neurons. *Bioessays*. 2006; 28: 23–34.
72. **de Bruyne M, Foster K, Carlson JR.** Odor coding in the *Drosophila* antenna. *Neuron*. 2001; 30: 537–52.
73. **Stensmyr MC, Giordano E, Balloi A, *et al.*** Novel natural ligands for *Drosophila* olfactory receptor neurones. *J Exp Biol*. 2003; 206: 715–24.
74. **Suh GS, Wong AM, Hergarden AC, *et al.*** A single population of olfactory sensory neurons mediates an innate avoidance behaviour in *Drosophila*. *Nature*. 2004; 431: 854–9.
75. **Scott K, Brady R, Cravchik A, *et al.*** A chemosensory gene family encoding candidate gustatory and olfactory receptors in *Drosophila*. *Cell*. 2001; 104: 661–73.
76. **Faucher C, Forstreuter M, Hilker M, *et al.*** Behavioral responses of *Drosophila* to biogenic levels of carbon dioxide depend on life-stage, sex and olfactory context. *J Exp Biol*. 2006; 209: 2739–48.
77. **Helenius IT, Krupinski T, Turnbull DW, *et al.*** Elevated CO2 suppresses specific *Drosophila* innate immune responses and resistance to bacterial infection. *Proc Natl Acad Sci USA*. 2009; 106: 18710–15.
78. **Bretscher AJ, Busch KE, de Bono M.** A carbon dioxide avoidance behavior is integrated with responses to ambient oxygen and food in Caenorhabditis elegans. *Proc Natl Acad Sci USA*. 2008; 105: 8044–9.
79. **Hallem EA, Sternberg PW.** Acute carbon dioxide avoidance in Caenorhabditis elegans. *Proc Natl Acad Sci USA*. 2008; 105: 8038–43.
80. **McGrath PT, Rockman MV, Zimmer M, *et al.*** Quantitative mapping of a digenic behavioral trait implicates globin variation in *C. elegans* sensory behaviors. *Neuron*. 2009; 12: 692–9.
81. **Bahn YS, Cox GM, Perfect JR, *et al.*** Carbonic anhydrase and CO_2 sensing during Cryptococcus neoformans growth, differentiation, and virulence. *Curr Biol*. 2005; 15: 2013–20.
82. **Sheth CC, Johnson E, Baker ME, *et al.*** Phenotypic identification of Candida albicans by growth on chocolate agar. *Med Mycol*. 2005; 43: 735–8.
83. **Sheth CC, Mogensen EG, Fu MS, *et al.*** Candida albicans HSP12 is co-regulated by physiological CO_2 and pH. *Fungal Genet Biol*. 2008; 45: 1075–80.
84. **Mitchell AP.** Fungal CO_2 sensing: a breath of fresh air. *Curr Biol*. 2005; 15: R934–6.
85. **Rocha CR, Schröppel K, Harcus D, *et al.*** Signaling through adenylyl cyclase is essential for hyphal growth and virulence in the pathogenic fungus Candida albicans. *Mol Biol Cell*. 2001; 12: 3631–43.
86. **Alspaugh JA, Pukkila-Worley R, Harashima T, *et al.*** Adenylyl cyclase functions downstream of the Galpha protein Gpa1 and controls mating and pathogenicity of Cryptococcus neoformans. *Eukaryot Cell*. 2002; 1: 75–84.
87. **Mogensen EG, Janbon G, Chaloupka J, *et al.*** Cryptococcus neoformans senses CO2 through the carbonic anhydrase Can2 and the adenylyl cyclase Cac1. *Eukaryot Cell*. 2006; 3: 103–11.
88. **Simon BA, Moody EJ, Johns RA.** Therapeutic gases: oxygen, carbon dioxide, nitric oxide, and helium. In: Brunton LL, Lazo JS, Parker KL, editors. Goodman & Gilman's the pharmacological basis of therapeutics. 11th ed. New York: The McGraw-Hill Companies, Inc.; 2006, pp. 387–400.
89. **Eaton D, Pooler J.** Regulation of hydrogen ion balance. In: Eaton D, Pooler J, editors. Vander's renal physiology. 7th ed. New York: The McGraw-Hill Companies, Inc.; 2009, pp. 150–79.
90. **Yucha C.** Renal regulation of acid-base balance (continuing education). *Nephrol Nurs J*. 2004 31: 201–8.
91. **Gutierrez G.** A mathematical model of tissue-blood carbon dioxide exchange during hypoxia. *Am J Respir Crit Care Med*. 2004; 169: 525–33.
92. **Levitzky M.** The control of breathing. In: Levitzky MG, editor. Pulmonary physiology. 7th ed. New York: The McGraw-Hill Companies, Inc.; 2007, pp. 49–56.
93. **Anthonisen NR, Wright EC, Hodgkin JE.** Prognosis in chronic obstructive pulmonary disease. *Am Rev Respir Dis*. 1986; 133: 14–20.
94. **Pauwels RA, Buist AS, Ma P, *et al.*** Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: National Heart, Lung, and Blood Institute and World Health Organization Global Initiative for Chronic Obstructive Lung Disease (GOLD): executive summary. *Respir Care*. 2001; 46: 798–825.
95. **Celli BR, Cote CG, Marin JM, *et al.*** The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med*. 2004; 350: 1005–12.
96. **Vitacca M, Bianchi L, Barbano L, *et al.*** Effects of acute on chronic respiratory failure on hypercapnia and 3-month survival. *Chest*. 2005; 128: 1209–15.
97. **Matthay M, Weiner-Kronish JP.** Intact epithelial barrier function is critical for the resolution of alveolar edema in humans. *Am Rev Respir Dis*. 1990; 142: 1250–7.
98. **Sznajder JI.** Alveolar edema must be cleared for the acute respiratory distress syndrome patient to survive. *Am J Respir Crit Care Med*. 2001; 163: 1293–4.
99. **Ware LB, Matthay MA.** Alveolar fluid clearance is impaired in the majority of patients with acute lung injury and the acute respiratory distress syndrome. *Am J Respir Crit Care Med*. 2001; 163: 1376–83.
100. **Mendez JL, Hubmayr RD.** New insights into the pathology of acute respiratory failure. *Curr Opin Crit Care*. 2005; 11: 29–36.
101. **Feihl F, Perret C.** Permissive hypercapnia. How permissive should we be? *Am J Respir Crit Care Med*. 1994; 150: 1722–37.
102. **Amato MB, Barbas CS, Medeiros DM, *et al.*** Effect of a protective-ventilation strategy on mortality in the acute respiratory distress syndrome. *N Engl J Med*. 1998; 338: 347–54.
103. **Laffey JG, Engelberts D, Kavanagh BP.** Injurious effects of hypocapnic alkalosis in the isolated lung. *Am J Respir Crit Care Med*. 2000; 162: 399–405.
104. **Broccard AF, Hotchkiss JR, Vannay C, *et al.*** Protective effects of hypercapnic acidosis on ventilator-induced lung injury. *Am J Respir Crit Care Med*. 2001; 164: 802–6.
105. **Laffey JG, Honan D, Hopkins N, *et al.*** Hypercapnic acidosis attenuates endotoxin-induced acute lung injury. *Am J Respir Crit Care Med*. 2004; 169: 46–56.
106. **Laffey JG, Kavanagh BP.** Carbon dioxide and the critically ill – too little of a good thing? *Lancet*. 1999; 354: 1283–6.
107. **Rodrigo C, Rodrigo G.** Subarachnoid hemorrhage following permissive hypercapnia in a patient with severe acute asthma. *Am J Emerg Med*. 1999; 17: 697–9.
108. **Mutlu GM, Factor P, Schwartz DE, *et al.*** Severe status asthmaticus: management with permissive hypercapnia and inhalation anesthesia. *Crit Care Med*. 2002; 30: 477–80.
109. **Laffey JG, Tanaka M, Engelberts D, *et al.*** Therapeutic hypercapnia reduces pulmonary and systemic injury following in vivo lung reperfusion. *Am J Respir Crit Care Med*. 2000; 162: 2287–94.
110. **Sinclair SE, Kregenow DA, Lamm WJ, *et al.*** Hypercapnic acidosis is protective in an in vivo model of ventilator induced lung injury. *Am J Respir Crit Care Med*. 2002; 166: 403–8.
111. **Strand M, Ikegami M, Jobe AH.** Effects of high PCO_2 on ventilated preterm lamb lungs. *Pediat Res*. 2003; 53: 468–72.

112. **Lang JD, Chumley P, Eiserich JP, *et al.*** Hypercapnia induces injury to alveolar epithelial cells via a nitric oxide-dependent pathway. *Am J Physiol Lung Cell Mol Physiol*. 2000; 279: L994–1002.
113. **Pfeiffer B, Hachenberg T, Wendt M, *et al.*** Mechanical ventilation with permissive hypercapnia increases intrapulmonary shunt in septic and nonseptic patients with acute respiratory distress syndrome. *Crit Care Med*. 2002; 30: 285–9.
114. **Vertrees RA, Nason R, Hold MD, *et al.*** Smoke/burn injury-induced respiratory failure elicits apoptosis in ovine lungs and cultured lung cells, ameliorated with arteriovenous CO_2 removal. *Chest*. 2004; 125: 1472–82.
115. **Doerr CH, Gajic O, Berrios JC, *et al.*** Hypercapnic acidosis impairs plasma membrane wound resealing in ventilator-injured lungs. *Am J Respir Crit Care Med*. 2005; 171: 1371–7.
116. **Lang JD, Figueroa M, Sanders KD, *et al.*** Hypercapnia *via* reduced rate and tidal volume contributes to lipopolysaccharide-inducedlung injury. *Am J Respir Crit Care Med*. 2005; 171: 147–57.
117. **Kregenow DA, Swenson ER.** The lung and carbon dioxide: implications for permissive and therapeutic hypercapnia. *Eur Respir J*. 2002; 20: 6–11.
118. **Chen J, Lecuona E, Briva A, *et al.*** Carbonic anhydrase II and alveolar fluid reabsorption during hypercapnia. *Am J Respir Cell Mol Biol*. 2008; 38: 32–7.
119. **Broden CC.** Acute renal failure and mechanical ventilation: reality or myth? *Crit Care Nurse*. 2009; 29: 62–75.
120. **Badre NH, Martin ME, Cooper RL.** The physiological and behavioral effects of carbon dioxide on *Drosophila* melanogaster larvae. *Comp Biochem Physiol A Mol Integr Physiol*. 2005; 140: 363–76.
121. **Barron AB.** Anaesthetising *Drosophila* for behavioural studies. *J Insect Physiol*. 2000; 46: 439–42.
122. **Kaiser M.** Influence of anesthesia by carbon dioxide on hatching time and weight at eclosion in *Drosophila* melanogaster. *Drosophila Inf Serv*. 1995; 76: 92–3.
123. **Perron JM, Huot L, Corrivault GW, *et al.*** Effects of carbon dioxide anaesthesia on *Drosophila* melanogaster. *J Insect Physiol*. 1972; 18: 1869–74.
124. **Nicolas G, Sillans D.** Immediate and latent effects of carbon dioxide on insects. *Annu Rev Entomol*. 1989; 34: 97–116.
125. **Roberts DB.** *Drosophila*: a practical approach. 2nd ed. New York: Oxford University Press, Inc.; 1998.
126. **Nilson TL, Sinclair BJ, Roberts SP.** The effects of carbon dioxide anesthesia and anoxia on rapid cold-hardening and chill coma recovery in *Drosophila* melanogaster. *J Insect Physiol*. 2006; 52: 1027–33.
127. **Mainguy V, Maltais F, Saey D, *et al.*** Peripheral muscle dysfunction in idiopathic pulmonary arterial hypertension. *Thorax*. 2009. DOI: 10.1136/thx.2009.117168
128. **Maltais F, Debigaré R.** Biology of muscle impairment in COPD. *Monaldi Arch Chest Dis*. 2003; 59: 338–41.
129. **Chen Y, Cann MJ, Litvin TN, *et al.*** Soluble adenylyl cyclase as an evolutionarily conserved bicarbonate sensor. *Science*. 2000; 289: 625–8.
130. **Zippin JH, Chen Y, Nahirney P, *et al.*** Compartmentalization of bicarbonate-sensitive adenylyl cyclase in distinct signaling microdomains. *FASEB J*. 2003; 17: 82–4.
131. **Zippin JH, Farrell J, Huron D, *et al.*** Bicarbonate-responsive "soluble" adenylyl cyclase defines a nuclear cAMP microdomain. *J Cell Biol*. 2004; 164: 527–34.
132. **Summers BA, Overholt JL, Prabhakar NR.** Augmentation of L-type calcium current by hypoxia in rabbit carotid body glomus cells: evidence for a PKC-sensitive pathway. *J Neurophysiol*. 2000; 84: 1636–44.
133. **Summers BA, Overholt JL, Prabhakar NR.** CO(2) and pH independently modulate L-type Ca(2+) current in rabbit carotid body glomus cells. *J Neurophysiol*. 2002; 88; 604–12.
134. **Overholt JL, Summers BA, Ficker E, *et al.*** CO_2/HCO_3- modulates K^+ and Ca^{2+} currents in glomus cells of the carotid body. *Adv Exp Med Biol*. 2001; 499: 61–6.
135. **Dorman PJ, Sullivan WJ, Pitts RF.** The renal response to acute respiratory acidosis. *J Clin Invest*. 1954; 33: 82–90.
136. **Eiam-ong S, Laski ME, Kurtzman NA, *et al.*** Effect of respiratory acidosis and respiratory alkalosis on renal transport enzymes. *Am J Physiol Renal Physiol*. 1994; 267: F390–9.
137. **Bichara M, Paillard M, Leviel F, *et al.*** Na:H exchange and the primary H pump in the proximal tubule. *Am J Physiol*. 1983; 244: F165–71.
138. **Gluck SL.** Acid sensing in renal epithelial cells. *J Clin Invest*. 2004; 114: 1696–9.
139. **Romero MF, Hediger MA, Boulpaep EL, *et al.*** Expression cloning and characterization of a renal electrogenic Na^+/HCO^3- cotransporter. *Nature*. 1997; 387: 409–13.
140. **Schwartz GJ, Al-Awqati Q.** Carbon dioxide causes exocytosis of vesicles containing H^+ pumps in isolated perfused proximal and collecting tubules. *J Clin Invest*. 1985; 75: 1638–44.
141. **Robertson HM, Kent LB.** Evolution of the gene lineage encoding the carbon dioxide receptor in insects. *J Insect Sci*. 2009; 9: 19.
142. **Clyne PJ, Warr CG, Carlson JR.** Candidate taste receptors in *Drosophila*. *Science*. 2000; 287: 1830–4.
143. **Robertson HM, Warr CG, Carlson JR.** Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila* melanogaster. *Proc Natl Acad Sci USA*. 2003; 100: 14537–42.
144. **Wistrand M, Kall L, Sonnhammer EL.** A general model of G protein-coupled receptor sequences and its application to detect remote homologs. *Protein Sci*. 2006; 15: 509–21.
145. **Wicher D, Schafer R, Bauernfeind R, *et al.*** *Drosophila* odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. *Nature*. 2008; 452: 1007–11.
146. **Sato K, Pellegrino M, Nakagawa T, *et al.*** Insect olfactory receptors are heteromeric ligand-gated ion channels. *Nature*. 2008; 452: 1002–6.
147. **Benton R, Sachse S, Michnick SW, *et al.*** Atypical membrane topology and heteromeric function of *Drosophila* odorant receptors *in vivo*. *PLoS Biol*. 2006: e20.
148. **Sun L, Wang H, Hu J, *et al.*** Guanylyl cyclase-D in the olfactory CO2 neurons is activated by bicarbonate. *Proc Natl Acad Sci USA*. 2009; 106: 2041–6.
149. **Hu J, Zhong C, Ding C, *et al.*** Detection of near-atmospheric concentrations of CO_2 by an olfactory subsystem in the mouse. *Science*. 2007; 317: 953–7.
150. **Wuttke MS, Buck J, Levin LR.** Bicarbonate-regulated soluble adenylyl cyclase. *JOP*. 2001; 2: 154–8.
151. **Hammer A, Hodgson DR, Cann MJ.** Regulation of prokaryotic adenylyl cyclases by CO_2. *Biochem J*. 2006; 396: 215–8.