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Phylogeny of CO_2/H^+ chemoreception in vertebrates

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Abstract

The traditional view has been that respiratory chemoreceptors responsive to changes in P_{CO_2}/pH first evolved in air breathing vertebrates at both peripheral and central sites. Recent evidence, however, suggests that fish also possess chemoreceptors responsive to changes in P_{CO_2} per se. In many species these receptors reside in the gills and respond primarily to changes in aquatic rather than arterial P_{CO_2}. There is also scattered evidence to suggest that central CO_2/H^+-sensitive chemoreceptors may be present in representatives of all fish groups but only the data for air breathing fish are strong and convincing. The phylogenetic trends that are emerging indicate that the use of CO_2 chemoreception for cardiorespiratory processes arose much earlier than previously believed, (arguably) that CO_2 chemoreception may first have arisen in the periphery sensitive to the external environment and that central CO_2/H^+ chemoreception subsequently arose multiple times in association with several of the independent origins of air breathing, and that the mechanisms of CO_2/H^+ chemotransduction may be as varied as the different receptor groups involved. © 2002 Published by Elsevier Science B.V.

Keywords: Carbon dioxide; respiratory chemoreceptors; fish; Control of breathing; chemoreceptors; vertebrates; Gill; fish; CO_2 chemoreceptors; peripheral and central

1. Introduction

There is a rich literature describing the ventilatory responses of vertebrates to hypercapnia and acidosis and this literature documents tremendous variability, reflecting adaptations of different species to environmental, behavioural and physiological demands (Shelton et al., 1986; Smatresk, 1990; Milsom, 1995; Tenney and Leiter, 1995). Much of this variability arises from the type, stimulus specificity and sensitivity of different chemoreceptors involved in eliciting these responses (Milsom, 1998). It is insightful in this context to examine the extent to which such variability arises due to adaptive trends involving a common receptor group versus phylogenetic trends in the occurrence of specific receptor groups. In this review I wish to focus on the latter and primarily address the question of when central CO_2/H^+ chemoreceptors first arose in the vertebrate lineage. By way of background, and to provide a phylogenetic context for this discussion, I will also briefly review the phylogenetic trends that are seen in the location, innervation and relative roles of peripheral CO_2/H^+ chemorecep-

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tors and will then go on to review the growing, and confusing literature on the occurrence of central chemosensitivity.

2. Phylogenetic trends in the location and innervation of peripheral CO_2/H^+ chemoreceptors

Due to the low capacitance of water for oxygen, water breathing vertebrates must produce a high flow rate of water across the gills to obtain sufficient O_2 to meet metabolic demands. Because of the high capacitance of water for CO_2, however, these high flow rates lead to rapid CO_2 excretion. The consequence of this difference in water capacitance for O_2 and CO_2 is that the arterial blood of most water breathers usually has very low CO_2 tensions (Pa_{CO_2}). These low levels of Pa_{CO_2} are accompanied by equally low [HCO_3^-] and, thus, pH levels which are only slightly alkaline by vertebrate standards. Regulation of Pa_{CO_2} and arterial pH (pHa) at these levels can be achieved by ion-transporting mechanisms that are largely independent of ventilation. Thus, not surprisingly, O_2 rather than CO_2/H^+ appears to exert the dominant chemical control over ventilation in water breathers (Dejours, 1973). In the phylogenetic progression from aquatic to terrestrial vertebrates, the switch from water to air as the respiratory medium, with its increase in O_2 capacitance, is accompanied by changes in the mean levels of Pa_{CO_2}, Pa_{O_2} and pHa, and an increase in the sensitivity of animals to $_{CO_2}/H^+$ as the primary respiratory stimulus at rest (Shelton et al., 1986). With this progression, the traditional view has been that respiratory chemoreceptors responsive to changes in P_{CO_2}/pH first evolved in air breathing vertebrates at both peripheral and central sites (Tenney and Leiter, 1995). Levels of arterial P_{CO_2} in water breathing fish are typically less than 2–3 Torr and it was assumed that the ventilatory responses of fish to changes in aquatic P_{CO_2}/pH were due to the effects of acidosis on haemoglobin oxygen transport (Smith and Jones, 1982). Recent data no longer support this view.

There is now good evidence to indicate that there are olfactory, and possibly gustatory receptors present in all vertebrates, including fish, that are sensitive to changes in CO_2/H^+ and that can influence ventilation (Ballam, 1984; Coates and Ballam, 1990; Coates et al., 1991; Kinkead and Milsom, 1996; Coates et al., 1996; Milsom et al., submitted for publication). In general, stimulation of these receptors has little effect on breathing in most species, but in some species, it inhibits breathing and this effect can be quite profound. Thus, in many mammals, 5% CO_2 has no effect on ventilation when it is only inhaled into the nasal passages (Sant'Ambrogio et al., 1984) while in some species of reptiles this is sufficient to completely inhibit ventilation (Ballam, 1985). Such a reflex inhibition of breathing may have arisen as a general defense response to exposure to harsh environmental stimuli, reducing CO_2 uptake while animals seek fresh air, and appears to have been up/down regulated in various species as a function of habitat (fossorial versus non-fossorial species) or behaviour (as a means of detecting predators/prey) (Ballam, 1985; Coates and Ballam, 1987).

There is also now strong evidence showing that some fish, at least, do possess peripheral chemoreceptors responsive to changes in P_{CO_2}/pH per se. These receptors reside primarily in the gills, are innervated by the glossopharyngeal and vagus nerves, and respond primarily to changes in aquatic rather than arterial P_{CO_2} (Reid et al., 2000; Sundin et al., 2000; McKendry et al., 2001; McKendry and Perry, 2001; Milsom et al., submitted for publication). Their distribution overlaps extensively with that of the gill O_2 chemoreceptors in fish and it is not yet clear whether both responses arise from the same sensory cells (Sundin et al., 1999; Reid et al., 2000; Sundin et al., 2000). These data suggest that chemoreceptors responsive to changes in P_{CO_2}/pH evolved much earlier than previously believed. They are present in representatives of the elasmobranches (McKendry et al., 2001; McKendry and Perry, 2001) and Actinopterygian fishes (Reid et al., 2000; Sundin et al., 2000) and it would appear that their initial role was to elicit cardiorespiratory responses to changes in the external environment. Complete branchial denervation does not eliminate the hypercarbic ventilatory response in all fish, however; indeed in the tench it does not

even reduce the response (Hughes and Shelton, 1962; Reid et al., 2000; Milsom et al., submitted for publication). This suggests that receptor sites outside the gills also exist that contribute to the ventilatory response to different degrees in different species. The presence/absence of similar receptors in lampreys and hagfishes (the Agnathans) remains an open question that bears further research.

Fig. 1 illustrates the primary locations of the (non-olfactory) peripheral CO_2/H^+-sensitive chemoreceptors identified in fish and other vertebrate groups. The location, innervation and homologies of these receptors have been reviewed recently, (Smatresk, 1990; Milsom, 1998) and this information will not be repeated here. Of note, however, are two observations. The first is that CO_2/H^+-sensitive chemoreceptors homologous to those found throughout the gill arches of fish are also found in association with the gas exchange surfaces, or blood vessels supplying the respiratory passages in all vertebrates except mammals (Fedde et al., 1977; Ishii et al., 1985, 1986; Scheid and Piiper, 1986; Douse et al., 1989). The presence of CO_2/H^+ chemoreceptors within the lungs of mammals responsive to changes in P_{CO_2} or CO_2 content of pulmonary arterial blood has been debated but never clearly established (Coleridge and Coleridge, 1986; Wasserman et al., 1986). Interestingly, while the presence of such receptors has been shown in these other groups, the physiological roles of the receptors associated with the pulmonary arteries in amphibians and turtles, and those found in the lungs of birds and non-chelonian reptiles (intrapulmonary chemoreceptors) remain unclear (Burger et al., 1974; Milsom et al., 1981; Fedde et al., 1982; Ishii et al., 1986; Coates and Ballam, 1987).

The other parallel observation of note is that the CO_2/H^+-sensitive chemoreceptors associated with the first gill arch of fish become the primary peripheral chemoreceptors in all other vertebrates. In fish, receptors in the first branchial arch are innervated by both the IXth and Xth cranial nerves (Reid et al., 2000; Sundin et al., 2000). In amphibians, and mammals, homologous receptors come to lie at the carotid bifurcation, innervated by the IXth cranial nerve (West and Van Vliet,

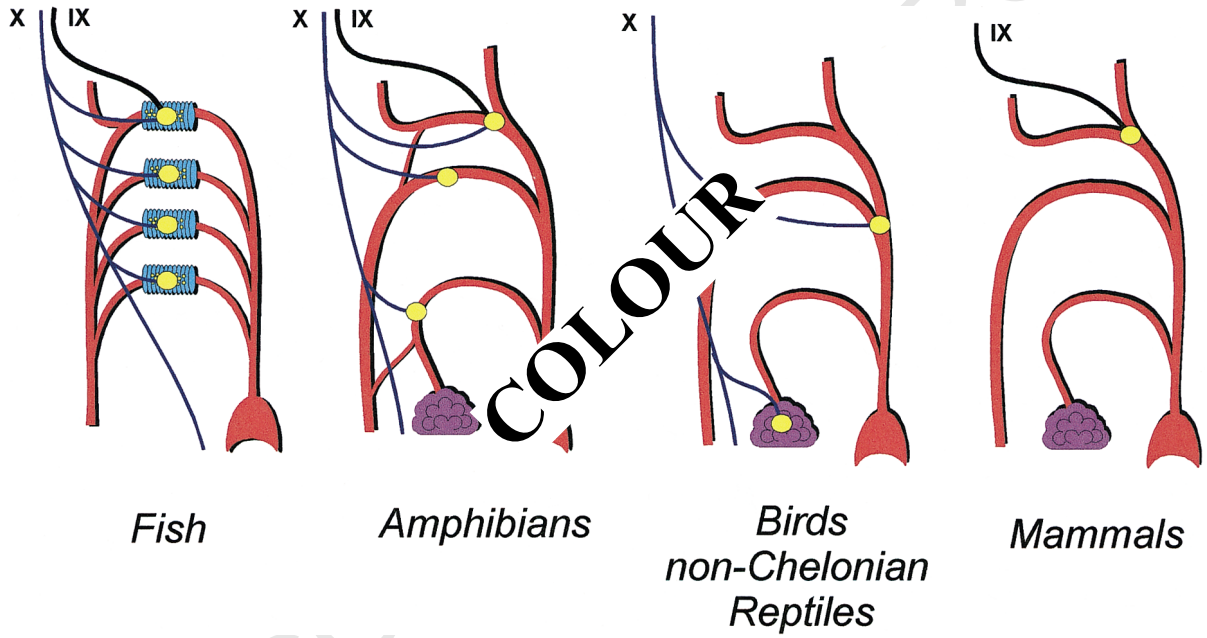

Fig. 1. Schematic diagram showing the distribution of peripheral arterial and pulmonary CO_2/H^+-sensitive chemoreceptors in various vertebrate groups. IX and X designate the IXth (glossopharyngeal) and Xth (vagus) cranial nerves.

1992), while in reptiles and birds they come to lie on the aorta and retain their innervation from the Xth cranial nerve (Abdel-Magied and King, 1978; West et al., 1981; Milsom, 1995). Just as has been previously described for O_2-sensitive receptors (Milsom, 1998), there is a trend to reduce the distribution of peripheral CO_2/H^+ receptors from multiple dispersed sites in fish and amphibia towards a single dominant receptor site in mammals. In conjunction with this, however, another chemoreceptive group arises that takes predominance over all others, the central chemoreceptors found within the brain itself.

Before turning to a consideration of the phylogenetic origins of the central chemoreceptors, note that peripheral CO_2/H^+ chemoreception would not appear to be a unique feature of any one cell type. The carotid body chemoreceptors are excited by CO_2 and act to stimulate ventilation (Gonzalez et al., 1994, 1995), olfactory receptors are excited by CO_2 and act to inhibit ventilation (Coates and Ballam, 1990), and intrapulmonary chemoreceptors are inhibited by CO_2 and this inhibition acts to depress ventilation (Scheid and Piiper, 1986). There is little known of the mechanisms by which changes in P_{CO_2}/H^+ alter chemoreceptor discharge but the evidence would suggest that several different mechanisms must exist.

3. Phylogenetic trends in the distribution and characteristics of central CO_2/H^+ chemoreceptors

Central CO_2/H^+ chemoreceptors have now been indirectly shown to exist in the adults of all tetrapod vertebrates. Thus, ventilatory responses to changes in the P_{CO_2}/pH of solutions perfusing either the ventriculocisternal spaces or the surface of the medulla have been described in frogs, toads, turtles and mammals (De Marneffe-Foulon, 1962; Hitzig and Jackson, 1978; Hitzig, 1982; Hitzig and Nattie, 1982; Loeschke, 1982; Hitzig et al., 1985; Smatresk and Smits, 1991; Branco et al., 1992; Nattie, 1990). Evidence for their presence in birds arises from studies of reflex ventilatory responses employing peripheral chemoreceptor denervation and brain cross-perfusions (Sebert, 1979; Milsom et al., 1981).

Partitioning relative roles to the central versus peripheral arterial chemoreceptors in producing ventilatory responses is not easy. The evidence suggests that the contribution of the arterial chemoreceptors varies both over the time course of the response and as a function of the magnitude of the stimulus. Many studies suggest that the carotid bodies in mammals contribute more to the initial phase of the hypercapnic ventilatory response than to the steady state changes (Black et al., 1971). It also appears that the carotid bodies only augment the drive of central respiratory neurons at moderate levels of hypercapnia (Heymans and Neil, 1958; Fidone and Gonzalez, 1986; Fitzgerald and Lahiri, 1986). The overall impression is that while the peripheral arterial chemoreceptors can contribute as much as 50% to the ventilatory response to small transient changes in P_{CO_2}/pH, under steady state conditions the central chemoreceptors are the dominant source of the chemosensory drive (Rodman et al., 2001). Thus, from a phylogenetic perspective, once central CO_2/H^+ chemoreceptors appear, they take on the predominant role of providing chemosensory drive under steady state conditions.

It has proven very difficult to identify specific central receptors responsible for eliciting reflex ventilatory responses in any group and, thus, the exact locations and discharge characteristics of these 'putative' central chemoreceptors remain unknown (Ballantyne and Scheid, 2000). In mammals, where central CO_2-chemosensitivity has been most extensively studied, CO_2-sensitive cells have been found at many locations within the brainstem (rostral and caudal ventrolateral surface of the medulla, nucleus tractus solitarii, fastigial nucleus, rostral ventral respiratory group, retrotrapezoid nucleus, medullary raphe and locus coeruleus), and stimulation of a small fraction of all central chemosensitive sites can result in a large ventilatory response (Coates et al., 1993; Nattie, 2000). It has also been shown that there are differences in the chemosensory mechanisms of cells sensitive to changes in pH on the caudal and rostral ventrolateral surfaces of the medulla (Nattie, 1986a,b; Okada et al., 1993). While imidazole-histidine is involved in the mechanism of central chemosensitivity in the rostral medulla, it

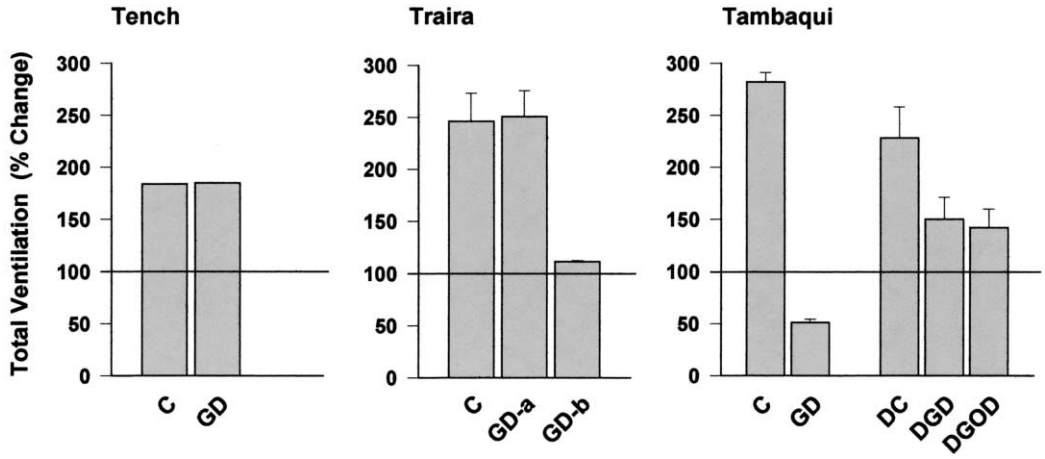

Fig. 2. Change (% change from normocapnic values) in total ventilation on exposure to hypercarbic water in the tench, traira and tambaqui. C, control; GD, gill denervated (a and b for traira represent subgroups of animals that responded differently to the same treatment, see text for explanation); DC, decerebrate control; DGD decerebrate and gill denervated; DGOD, decerebrate and gills and orobranchial cavity denervated. (Data from Hughes and Shelton, 1962 (tench), Reid et al., 2000 (traira) and Milsom et al., submitted for publication (tambaqui)).

is not in the caudal medulla. It would appear that different cells at different sites have become CO_2/H^+ chemosensitive utilizing different mechanisms for signal transduction. Thus, in tetrapod vertebrates, there may be multiple central sites for CO_2/H^+ chemoreception and, just as in the periphery, central CO_2/H^+ chemoreception may not be a unique feature of any one cell type.

4. Phylogenetic origin of central CO_2/H^+ chemoreceptors—water breathing fish

Given the new evidence to suggest that fish do indeed possess peripheral chemoreceptors responsive to changes in CO_2/H^+ per se, it is worth revisiting the evidence relevant to the question of whether fish possess central CO_2/H^+ chemoreceptors. Much of the early evidence to suggest that this might be the case arose from gill denervation studies. In many species of fish, the gills are the predominant site of O_2 chemoreception (Smatresk, 1990; Burleson et al., 1992) but in numerous studies, total gill denervation failed to eliminate the ventilatory response to hypercarbia or acidosis (Hughes and Shelton, 1962; Reid et al., 2000; Milsom et al., submitted for publication). Fig. 2 summarizes data from three such studies to illustrate the variability that can be seen in the data. In the tench (*Tinca tinca*), total denervation of the IXth and Xth cranial nerve supply from the gills led to a decrease in breathing frequency and an increase in the amplitude of mouth and opercular movements but had little or no effect on the hypercarbic (20 mmHg P_{CO_2}) ventilatory response (a small reduction in breathing frequency accompanied by a large increase in ventilation amplitude) (Hughes and Shelton, 1962) (Fig. 2, left panel). In traira (*Hoplias malabaricus*), total gill denervation completely eliminated the response to hypercarbia (5% CO_2) in five of seven fish studied (GD-b in middle panel, Fig. 2) but had no effect on total ventilation in the other two (it reduced the frequency response but dramatically elevated the amplitude response) (Reid et al., 2000) (GD-a in middle panel, Fig. 2). Finally, in tambaqui (*Colosomma macropomum*), while gill denervation reversed the hypercarbic (5% CO_2) ventilatory response in animals with an intact central nervous system, it only reduced the response in decerebrate fish (the remaining response was due to an increase in breathing frequency) (Milsom et al., submitted for publication) (Fig. 2, right panel). Species differences aside, one

conclusion that can be drawn from these studies is that gill denervation can have highly varied effects on different components of the ventilatory response (frequency versus amplitude) under different conditions. The other is that there are receptors outside the gills, at least in some fish, that can give rise to ventilatory responses to changes in CO_2/H^+.

The strongest evidence to suggest that the extra-branchial receptors might be central CO_2/H^+ chemoreceptors is perhaps some of the oldest evidence. Hughes and Shelton (1962) explored this question in the tench after discovering that gill denervation did not eliminate the hypercarbic ventilatory response (Fig. 2). In one fish, they injected small boluses (0.2–1 μl) of pH 7.6 solution (acidic for a fish) into areas of the posterior medulla (caudal to the facial lobe) from which electrical discharges in rhythm with respiration were obtained, and found sites where injections could increase the amplitude of respiratory movements (the normal hypercarbic ventilatory response in tench) as well as cause cessation of all movements with the mouth closed or partial cessation with the mouth open. They concluded that while these data could not account for the normal hypercarbic ventilatory response of the fish, they did indicate that there were chemosensitive cells within the medulla. Rovainen (1977) also explored this question using the isolated brain of the lamprey. He found that increasing the bicarbonate concentration (concentration not given) of the mock csf used to perfuse this preparation decreased the frequency of fictive respiratory motor output in 4 of 7 animals while titration of half the bicarbonate in normal mock csf with 1 mM HCl sometimes (3 of 8 animals) increased it.

Other attempts to demonstrate a role for central chemoreceptors in regulating water breathing in fish have not been successful. In one elegant series of studies, it was demonstrated that while the skate responded strongly to hypercarbia (Graham et al., 1990), the increase in ventilation did not correlate well with changes in Pa_{CO_2}, csf pH or the pHi of brain tissue (^{14}C-DMO method) (Wood et al., 1990). The best correlation was with

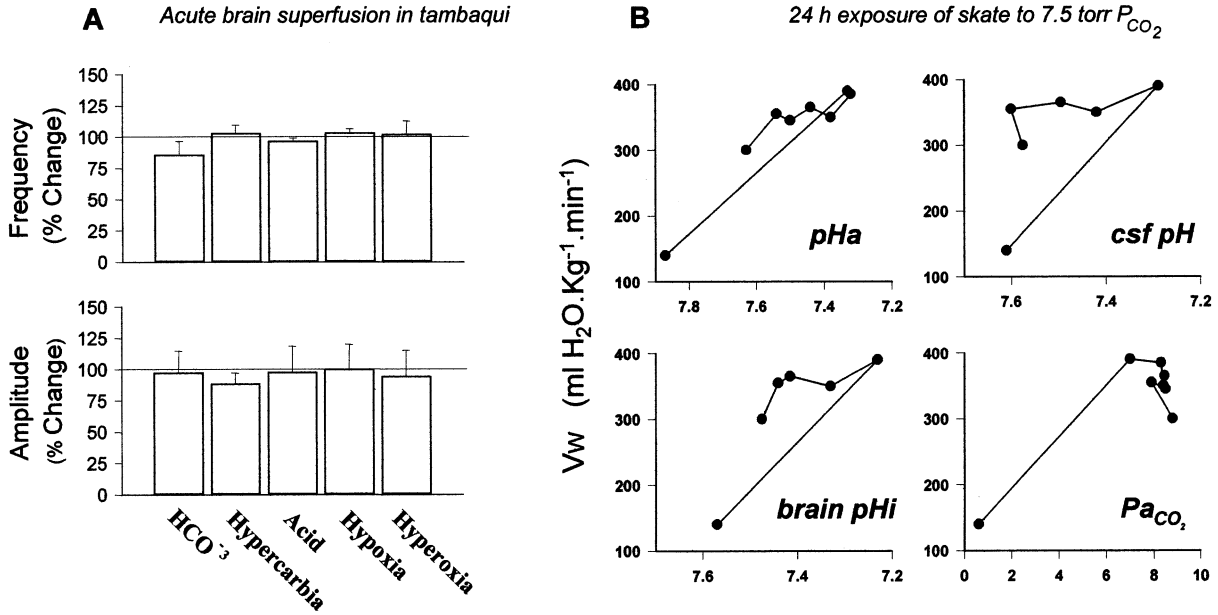

Fig. 3. (A) Effects on breathing frequency and amplitude of acute brain superfusion of decerebrate tambaqui with different solutions (Data from Milsom et al., submitted for publication). (B) Effects of 24 h exposure of the skate to 7.5 Torr P_{CO_2} on total gill ventilation (VW) as a function of the changes in arterial P_{CO_2} (Pa_{CO_2}), and the pH of arterial blood (pHa), the pH of cerebrospinal fluid (csf pH) and brain intracellular pH (pHi). (Data from Wood et al. (1990)).

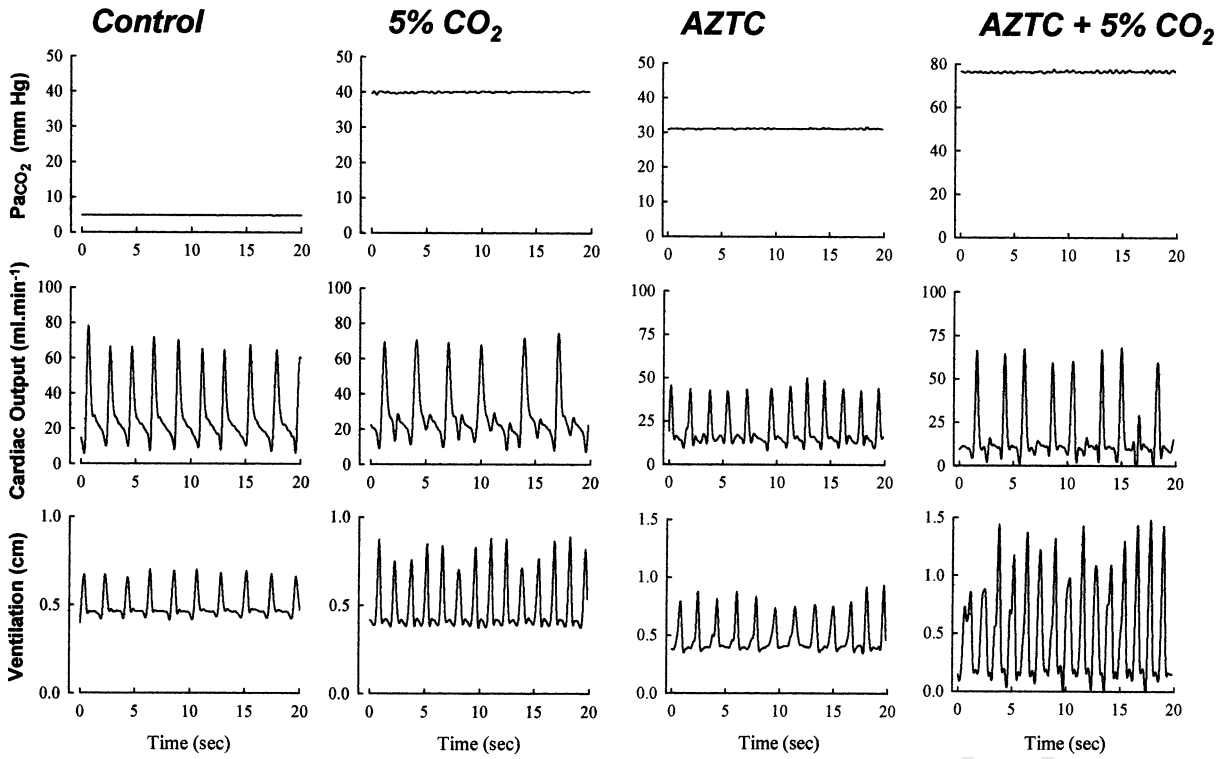

Fig. 4. Arterial P_{CO_2} (Pa_{CO_2} in mmHg), cardiac output (Vb in ml/min) and ventilation (opercular movement in cm) recorded in control animals exposed to normocarbic water (Control), animals exposed to water containing 5% CO_2 (5% CO_2), animals in normocarbic water after treatment with acetazolamide (AZTC) and animals pretreated with acetazolamide during exposure to water containing 5% CO_2 (AZTC + 5% CO_2). (unpublished data from Perry, Milsom, Reid, Rantin and Gilmour).

the pH of the arterial blood (Wood et al., 1990) (Fig. 3B). Further studies by other investigators that either perfused the cerebral ventricles or superfused the brainstem with acidic, alkalotic or hypercapnic mock csf in amia, trout, and tambaqui (Fig. 3A) were without effect (Hedrick et al., 1991; Burleson et al., 1992; Milsom et al., submitted for publication) (Fig. 3).

Most recently, the distribution of CO_2/H^+ chemoreceptors has been examined closely in the tambaqui (*Colosomma macropomum*), a neotropical teleost fish. In this species, approximately 25% of the increase in breathing frequency elicited by hypercarbic exposure remained in decerebrate fish after complete denervation of the gills and extensive denervation of the orobranchial cavity (Fig. 2, right panel) (Milsom et al., submitted for publication). The remaining response did not appear to arise centrally since superfusion of the brainstem of these fish with acidic and/or alkalotic solutions was without effect (Fig. 3B) (Sundin et al., 2000). The brainstem was still perfused with normocapnic blood under these conditions, however, and so it was possible that central receptors existed that were insensitive to external csf but sensitive to internal pH. However, we also found that injection of acetazolamide into these fish led to significant retention of CO_2 without any effect on ventilation. The animals were still capable of responding to aquatic hypercarbia indicating that the acetazolamide had not affected chemoreceptor function (Fig. 4). We also found that when animals exposed to aquatic hypercarbia were rapidly reintroduced to fresh water, ventilation immediately returned to normal despite the fact that arterial P_{CO_2} remained elevated for quite some time. Finally we found that while the fish demonstrated a brisk response to a bolus injection of

CO_2 equilibrated water into the mouth (40 ml of 10% CO_2 equilibrated water), they did not respond to bolus injections of CO_2 equilibrated saline injected into either the caudal vein or the ventral aorta (1.33 ml of 20% CO_2 equilibrated saline) (ventilation/perfusion in fish is roughly 30/1 and injection volumes were chosen accordingly) (Perry, Milsom, Reid, Rantin and Gilmour, in preparation). All of these data indicate that the normal hypercarbic response of this species is to changes in the CO_2 of the external environment and provide no evidence for the existence of central chemoreceptors.

The most parsimonious explanation of the data accumulated to date is that while water breathing fish do exhibit a hypercarbic ventilatory response, per se, the response arises from peripheral receptors (possibly found throughout the orobranchial cavity) sensitive primarily to changes in the external environment. Attempts to seek parsimony, however, assume phylogenetic continuity and species similarity rather than species differences which may not be the case as the following discussion will attest.

5. Phylogenetic origin of central CO_2/H^+ chemoreceptors—bimodal and air breathing fish

Exposure to aquatic hypercarbia stimulates air breathing in most air breathing fishes, but not all. In general, gill breathing is also stimulated by low levels of aquatic hypercarbia but the effect is relatively small and progressive hypercarbia generally inhibits branchial ventilation (Johansen, 1970; Shelton et al., 1986; Smatresk, 1988). The level of aquatic CO_2 required to inhibit gill ventilation ranges from 2 to 10% in different species as does the level required to stimulate air breathing (Johansen et al., 1967, 1968; Johansen and Lenfant, 1968; Johansen et al., 1970; Hughes and Singh, 1970, 1971; Singh and Hughes, 1973; Lomholt and Johansen, 1974; Graham, 1976; Graham et al., 1977; Burggren, 1979; Smatresk and Cameron, 1982). In any given species, air breathing is stimulated before gill ventilation ceases (Jesse et al., 1967; Hughes and Singh, 1970; Graham et al., 1977). These thresholds can be affected by the level of aquatic P_{O_2} (Jesse et al., 1967; Hughes and Singh, 1970).

Aerial hypercarbia has no effect on ventilation in many air breathing species but does stimulate ventilation in others (Johansen et al., 1967, 1968; Lomholt and Johansen, 1974; Burggren, 1979). The nature of the response appears to depend on the efficacy of gill ventilation in eliminating CO_2. In species in which branchial CO_2 excretion is rapid, inhalation of CO_2 or injection of CO_2 into the air breathing organ may not result in any change in arterial P_{CO_2}. All of the CO_2 taken up by the air exchanger is eliminated by the aquatic exchanger. The implication of these data is that changes in gill and lung ventilation only occur if changes occur in P_{CO_2}/pH at the gills or in the arterial circulation.

Several recent reports suggest that in some species of air breathing fish, central CO_2/H^+ chemoreceptors may contribute to these responses. These studies employed in vitro brainstem-spinal cord preparations of the holostean fish, the gar (*Lepisosteus osseus*) and the teleost fish, the siamese fighting fish (*Beta splendens*). In these studies, fictive motor output identified as motor output that would produce 'air breathing' in vivo, was shown to be sensitive to changes in superfusate CO_2/H^+ (Fig. 5). While the evidence presented to suggest that this activity is fictive air breathing is relatively weak, subsequent superfusion of isolated brainstems with high CO_2/low pH solutions did lead to an increase in this motor output but had no effect on fictive gill ventilation (Wilson et al., 2000; Harris et al., 2000). However, most gar in normocarbic water do not air breathe and when exposed to hypercarbic waters will increase air breathing to only 6–7 breaths/h slowly over an 8 h period (Smatresk and Cameron, 1982) (Fig. 5). They can increase air breathing up to 35 breaths/h immediately following exhaustive exercise in association with a sustained acidosis (pH 7.2) (Burleson et al., 1998) suggesting that under some conditions responses may be larger and faster. With the in vitro preparation, on the other hand, fictive breathing at normal pH was roughly 130 breaths/h rising to over 250 breaths/h when the superfusate pH was reduced from 8.0 to 7.5 (Wilson et al., 2000). Thus, the fictive breathing

rates of the hypercapnic, in vitro preparations were more than 10–35 times the rates reported for intact animals while the normocapnic preparations discharged prolifically under conditions when they should have been silent (Fig. 5). This comparison is not straight forward, however, since in one study pH changes were produced in the external environment (Smatresk and Cameron, 1982) while in the other they were made centrally, near the putative chemoreceptor sites (Wilson et al., 2000). Furthermore, the resting pH at the receptor site may have been much lower than 8.0 under resting conditions in the in vitro preparation since the brain tissue is not perfused, and there are diffusion limitations in this preparation. In the only other holostean fish studied to date (the bowfin, *Amia calva*), superfusion of the brainstem of intact fish with acidotic and alkalotic solutions had no effect on ventilation (Hedrick et al., 1991). On the other hand, it has also been reported recently, that the South American lungfish (a Sarcopterygian fish belonging to the lineage giving rise to higher vertebrates), does respond well to perfusion of the IVth cerebral ventricle with mock csf of differing pH (Sanchez et al., submitted for publication) (Fig. 5).

It is clear that we still have too little data for too few species to draw firm conclusions and that the data we do have suggesting that fish possess central CO_2 chemosensitive cells for all but the Sarcopterygian fishes (the true lung fish) are equivocal. As a consequence, several interpretations of the data are possible (Fig. 6). If central chemoreceptors are present in the lamprey, the data would suggest that central CO_2 receptors predate the origin of the Agnatha and Elasmobranchs, and perhaps have been secondarily lost in some species. If they are only present in some fish which exhibit various forms of air breathing, then the possibility exists that they have arisen multiple times, in association with the evolution of air breathing. It is well established that air breathing has arisen independently many times in fishes. Finally, until it can be shown that the responses of some Actinopterygian fishes to changes in csf pH are physiologically relevant, the

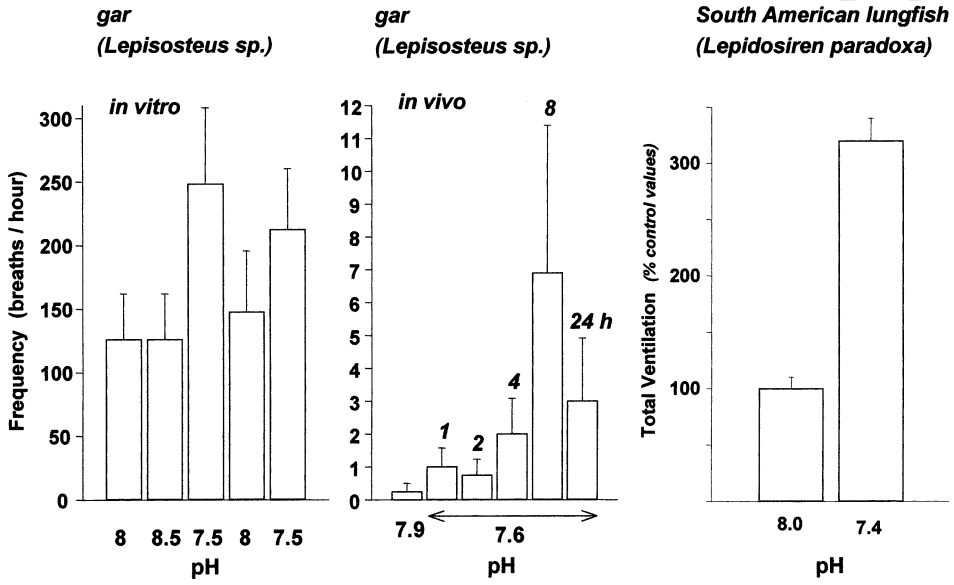

Fig. 5. Left panel: Effects on fictive air breathing frequency of superfusion of an in vitro brainstem-spinal cord preparation of the gar with solutions of varying pH (redrawn from Wilson et al., 2000). Middle panel: Effects on air breathing frequency of transferring intact gar from water of pH 7.9 to water of pH 7.6 over a 24 h period (Data from Smatresk and Cameron (1982)). Right panel: Effects on air breathing of superfusion of the IVth cerebral ventricle of South American lung fish with solutions of differing pH (Data from Sanchez et al., submitted for publication).

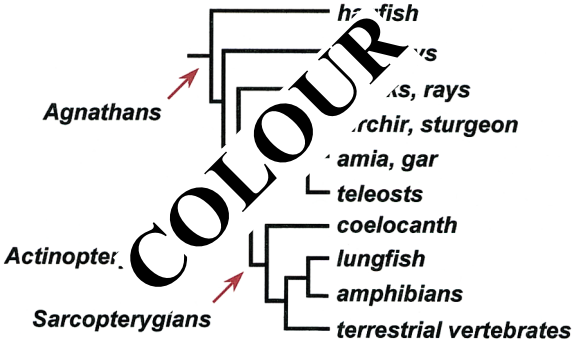

Fig. 6. Cladogram illustrating the phylogenetic relationships between various vertebrate groups. Arrows indicate branch points at which central CO_2/H^+-sensitive chemoreceptors may have arisen (see text for details).

possibility remains that central CO_2 receptors are only present in the true lung fish and arose only once in the line giving rise to Sarcopterygian fish, amphibians and terrestrial vertebrates.

An intriguing and related finding is that central chemosensitivity appears to develop slowly in amphibian tadpoles. It is not present in young tadpoles but develops over time. Using an in vitro preparation of the brainstem-spinal cord from tadpoles, it has been shown that while central CO_2/H^+-sensitive receptors initially stimulate gill ventilation in tadpoles, the net response (arising from the same or a different group of receptors) is transfered to lung ventilation just prior to metamorphosis from the aquatic larval stage into the air breathing adult form (Torgerson et al., 1997) (Fig. 7). In association with this, the primary location of the receptors in the brainstem shifts from a diffuse distribution to a rostral concentration in association with a rostral translocation of the sites essential for respiratory rhythmogenesis (Torgerson et al., 2001).

6. Conclusions

The sum of the data support the existence of CO_2/H^+-sensitive receptors in fish. Unfortunately a clear and complete picture of where these receptors are located has yet to emerge. It is tempting to speculate that CO_2-sensitive receptors first arose in the periphery, sensitive to the external environment, and that central chemoreceptors arose later in association with some of the multiple experiments in air breathing. This view is consistent with data from terrestrial vertebrates indicating that CO_2/H^+-sensitive chemoreceptors have arisen multiple times at multiple sites (both peripherally and centrally) employing different mechansims. Much more work will be required, however, before any firm conclusions can be drawn.

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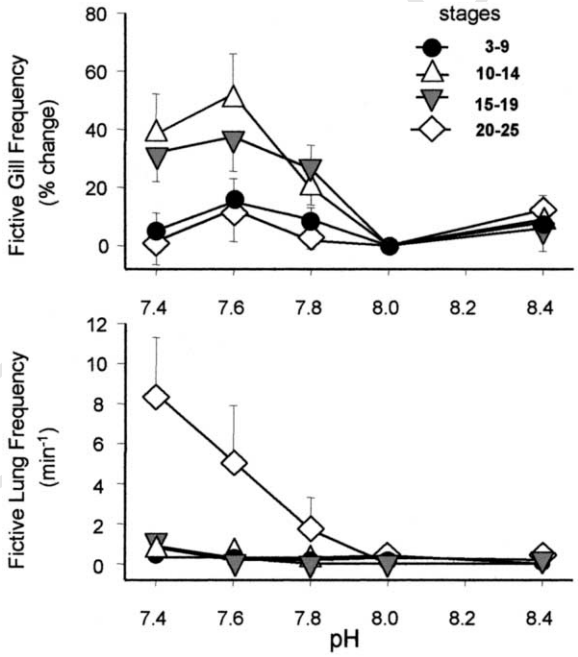

Fig. 7. Effects on fictive gill and air breathing frequencies of superfusion of in vitro brainstem-spinal cord preparations from bullfrog tadpoles (*Rana catesbeiana*) at different developmental stages with solutions of varying pH. (Data from Torgerson et al. (1997)).

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