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Evidence of a role for catecholamines in the control of breathing in fish

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Contents

Respiratory rhythm generation in the CNS

Respiratory gas exchange in fish takes place by diffusion over the surfaces of the gills. Water is propelled continuously over the gills by the ventilatory muscles that operate around the jaws and skeletal elements in the gill arches, lining the pharynx (Ballintijn and Hughes, 1965; Hughes and Ballintijn, 1965). These muscles are innervated by cranial nerves with their neurone cell bodies located in the brainstem. Central recording and marking techniques have identified a longitudinal strip of neurones with spontaneous, respiration-related bursting activity, extending dorso-laterally throughout the whole extent of the medulla (Shelton, 1970; Waldron, 1972). These neurones comprise the trigeminal Vth, facial VIIth, glossopharyngeal IXth and vagal Xth motor nuclei, which drive the respiratory muscles, together with the descending trigeminal nucleus and the reticular formation (Ballintijn, 1982). Neural tract tracing by retrograde intra-axonal transport of horseradish peroxidase (HRP) along nerves innervating the respiratory muscles revealed that the neurones in the various motor nuclei are distributed in a sequential series in the brainstem of the dogfish, (*Scyliorhinus canicula*, Scylloidea) (Withington-Wray *et al.*, 1986; Levings and Taylor, 1987; Levings, 1990).

All the respiratory motor nuclei are interconnected; each receives an afferent projection from the descending trigeminal nucleus and has efferent and afferent projections to and from the reticular formation, which in turn has reciprocal connections with areas in the midbrain such as the mesencephalic tegmentum (Fig. 1). Rhythmic ventilatory

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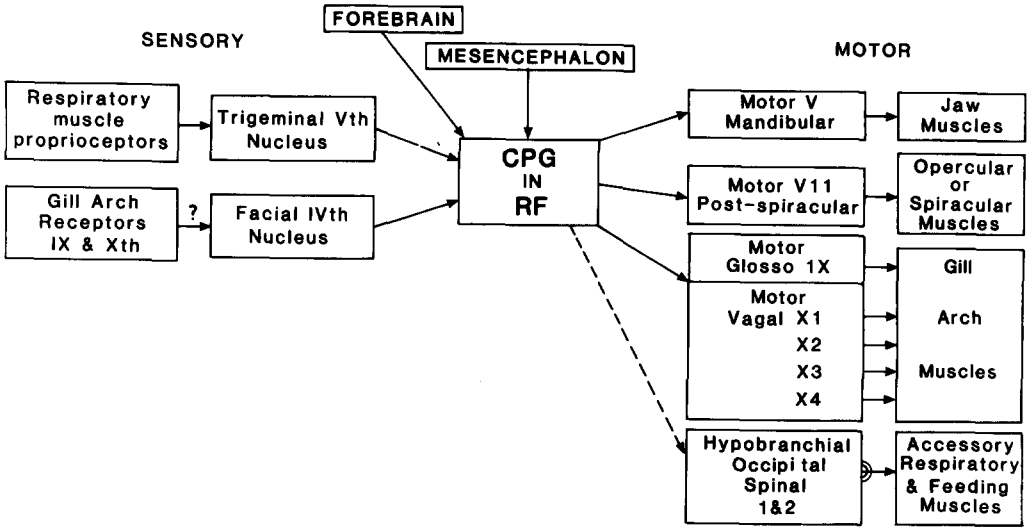


Fig. 1. Simple flow diagram of the possible functional connections involved in the central nervous control of ventilation in fish. Sensory inputs are represented on the left of the diagram and motor outputs to the respiratory muscles are on the right. The output to the hypobranchial nuclei is represented as a broken line to imply occasional recruitment by the CPG during forced ventilation. (Abbreviations: CPG, central pattern generator; RF, recticular formation.) (Reproduced with permission from Taylor, 1989.)

movements continue in fish following brain transection to isolate the medulla oblongata, although changes in pattern indicate that there are influences from higher centres (Shelton, 1959). The respiratory rhythm is thought to originate in a diffuse central pattern generator (CPG) in the reticular formation, which remains functional following anaesthesia (Ballintijn, 1987). The intermediate facial nucleus, which projects to the motor nuclei, also receives vagal afferents from the gill arches that innervate a range of tonically and phasically active mechanoreceptors. Activity in the CPG is modulated by these peripheral receptors located around the gill arches and jaws (Ballintijn, 1982; 1987). Part of the population of motoneurones innervating the respiratory muscles is silent in the paralysed animal in both fish and mammals, and may be stimulated to fire by artificially induced mechanoreceptive information (Ballintijn, 1982). The recruitment of these silent motoneurones may serve to increase motor output and consequently the amplitude of contraction of respiratory muscles. In addition, fish may recruit feeding muscles, innervated by the hypobranchial nerve trunk, which contains fibres from the occipital and anterior spinal motor nuclei (Fig. 1). These insert on the skeletal elements around the mouth and pharynx and can contribute to active, forced ventilation when respiratory demand is high (Ballintijn and Jüch, 1964; Hughes and Ballintijn, 1965).

Thus, rhythmic contractions of the respiratory muscles are determined by a central respiratory pattern generator (possibly located in the reticular formation) which is modulated by feedback on the force of ventilatory contractions from mechanoreceptors in the respiratory apparatus and by inputs from elsewhere in the CNS.

Chemoreceptor responses

Fish pump water over their gills at rates controlled with respect to oxygen supply or demand. In teleost fish, ventilation increases when oxygen supply is reduced by environmental hypoxia, or when transport in the blood is reduced – either directly by anaemia or indirectly by hypercapnia, when the resultant acidosis causes a Root effect, reducing oxygen-carrying capacity (reviews: Randall, 1982, 1990). Similarly, an increase in oxygen demand during vigorous swimming or following the stress of experimental manipulation results in an increase in ventilation rate. Obversely, an increase in oxygen supply resulting from environmental hyperoxia causes a decrease in ventilation rate, which may result in hypoventilatory hypercapnia (Fig. 2).

These responses to hypoxia can be interpreted as arising from the stimulation of peripheral chemoreceptors. There appear to be two types of chemoreceptors located on the gills of teleost fishes: external chemoreceptors monitoring the partial pressure of oxygen in the water and mediating hypoxic cardiovascular reflexes, and internal chemoreceptors monitoring blood oxygen supply (i.e. content × flow) and mediating changes in gill ventilation (Randall, 1982; Burleson and Smatresk, 1990a). Denervation of these latter chemoreceptors abolished the ventilatory responses of channel catfish (*Ictalurus punctatus*, Ictaluridae) to hypoxia and cyanide injection (Burleson and Smatresk, 1990b). In addition, venous oxygen receptors may be involved in the cardiac responses to hyperoxia (Barrett and Taylor, 1984). Evidence for the location and characteristics of these oxygen receptors is largely circumstantial, and the mechanisms by which reflex ventilatory changes are initiated are not clear (Taylor, 1985). Both the ventilatory and cardiac responses to sudden exposure to hypoxia or exercise are rapid in onset, indicating that nervous pathways are involved. The motor arm of the response is of course nervous, as the ventilatory muscles are innervated by the efferent cranial nerves and the heart is innervated by the

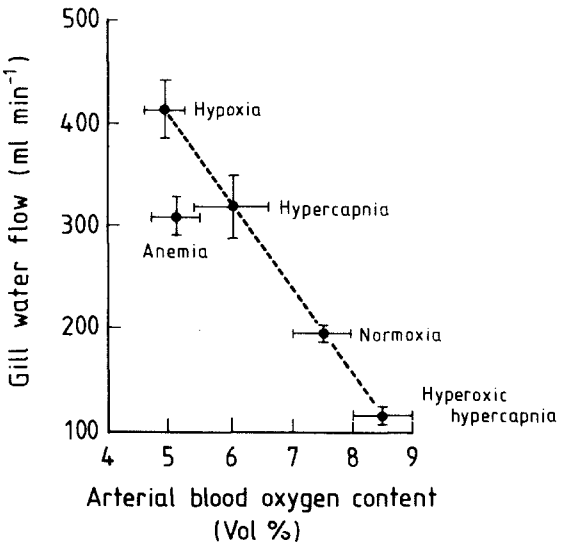


Fig. 2. Relationship between rate of water flow over the gills and the oxygen content of arterialized dorsal aortic blood from the rainbow trout, *Oncorhynchus mykiss* (mean values ± SEM). (Redrawn from Randall, 1982.)

parasympathetic vagus and in teleosts has in addition a sympathetic supply (Taylor, 1985). The afferent arm has not yet been clearly identified and neither the neuropharmacology of the central connections implicated in these reflex responses, nor the possible roles for circulating hormones, are known. Circulating catecholamines (chiefly noradrenalin and adrenalin) may be involved in the control of ventilation; this review now concentrates on this point.

Blood catecholamine levels in fish

Teleost fish have low (< 0.5 nmol l^{-1}), but measurable, resting levels of circulating catecholamines (Boutilier *et al.*, 1988). The measured levels are higher (above 20 nmol l^{-1}) in cannulated dogfish, possibly because they lack sympathetic innervation of the heart and branchial (gill) apparatus, so that circulating catecholamines may have an increased role in cardiovascular and ventilatory control (Butler *et al.*, 1978). However, these fish may have been stressed by cannulation. Changes in physiological state, particularly those induced by stressful stimuli such as physical disturbance (Nakano and Tomlinson, 1967), cause an increase in circulating catecholamine levels. Other stimuli include hypoxia (Butler *et al.*, 1978; Boutilier *et al.*, 1988) anaemia (Iwama *et al.*, 1987), hypercapnia (Perry and Kinkead, 1989; Perry *et al.*, 1989), acid infusion (Boutilier *et al.*, 1986) and violent exercise (Primmett *et al.*, 1986).

Circulating catecholamines play a number of important roles in the control of metabolism and respiratory gas exchange and transport in fish, including (a) stimulation of anaerobic glycolysis during exercise, (b) increased contractility of the heart, with a resultant increase in cardiac output, in vasodilation or in vasoconstriction of peripheral blood vessels, leading in turn to changes in blood pressure and vascular resistance to blood flow (Wood, 1976), (c) increased permeability of the gill respiratory epithelium (Isaia *et al.*, 1978) and (d) release of red blood cells from the spleen, increased red cell volume and increased intracellular pH (see Randall, 1990 for references).

It is now clear that catecholamine levels in the blood of fish are elevated during periods when oxygen delivery to the tissues may be compromised, for example following severe exercise, when the associated acidotic state may reduce blood oxygen content via the Root shift (Primmett *et al.*, 1986). The combination of their metabolic and physiological effects serves to improve oxygen supply. Many stimuli causing ventilatory increases, such as hypoxia (Thomas *et al.*, 1988), anaemia (Iwama *et al.*, 1987) and hypercapnia (Perry *et al.*, 1989), as illustrated in Fig. 2, are accompanied by increased levels of circulating catecholamines (Fig. 3). The simplest interpretation of this observation is that the ventilatory changes are in some way induced or reinforced by changes in circulating catecholamine levels.

Catecholamines and ventilation

Increases in circulating catecholamines are associated with either hyperventilation or hypoventilation in a wide variety of vertebrates via either peripheral or central reflex mechanisms (Peyraud-Waitzenegger, 1979; Folgering, 1980; Folgering *et al.*, 1982; Eldridge *et al.*, 1985; Jansen *et al.*, 1986; Taylor and Randall, 1990). In rabbits (Milsom and Sadig, 1983), cats (Mulligan *et al.*, 1986) and foetal lambs (Jansen*et al.*, 1986), catecholamines cause a β-adrenergic stimulation of the carotid and aortic bodies, which

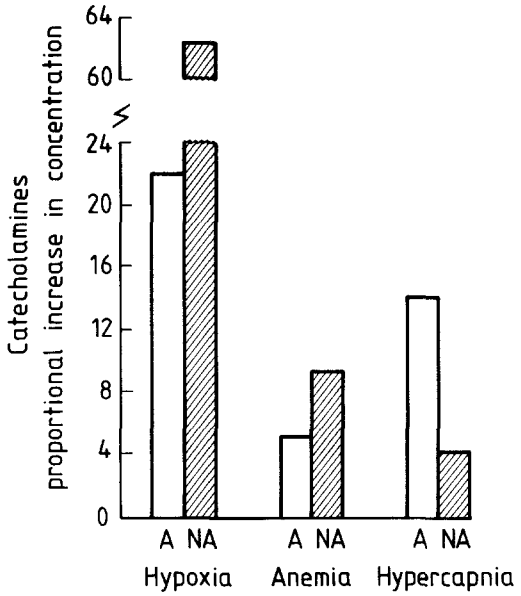


Fig. 3. The proportional increase in the plasma levels of circulating catecholamines in the rainbow trout elicited by exposure to hypoxia, hypercapnia or experimentally induced anaemia. (Abbreviations: A, adrenalin; NA, noradrenalin.) (Redrawn from Taylor and Randall, 1990.)

according to Mulligan *et al.*, (1986) is not an integral part of the process of O_2 chemoreception and transduction. The β-adrenergic blockers propranolol and atenolol reduced the afferent nervous activity recorded from the arterial chemoreceptors of the anaesthetized cat and reduced their sensitivity to increased potassium concentrations, which mimicked the effects of exercise (Paterson and Nye, 1988). Hudgel *et al.* (1986), however, reported that the β-adrenergic stimulation of ventilation in the goat was unaffected by denervation of the carotid body. They could find no evidence of functional β-adrenergic activity in the carotid body and yet there was a β-adrenergic stimulation of ventilation. Eldridge and Gill-Kumar (1980) concluded that peripheral mechanisms were not responsible for the increased ventilation observed following isoproterenol infusion into cats that persisted even after denervation of peripheral chemoreceptors. In a later study, Eldridge *et al.* (1985) found that isoproterenol infusion resulted in an acidosis in the extracellular cerebral fluid which could have made a small contribution to the observed stimulation of ventilation in glomectomized cats. The acidosis was considered to be due to an isoproterenol-mediated increase in neuronal metabolism but was insufficient to account for the entire increase in ventilation, so Eldridge *et al.* (1985) concluded that there could be a direct stimulation of brainstem neurones by isoproterenol, but the exact site was unknown. However, adult mammals and even the near-term fetal lambs of Jansen *et al.* (1986) have a complete blood-brain barrier to catecholamines (Evans *et al.*, 1974) such that no central action of circulating catecholamines would be expected. Indeed the catecholamine response in fetal lambs was blocked by denervation of the carotid sinus. The question of how isoproterenol crosses the apparently complete blood–brain barrier of mammals was not discussed by Eldridge *et al.* (1985). The barrier is thought to be incomplete in fetal mammals and it is possible that central effects of

catecholamines could dominate, or at least act synergistically with, peripheral effects in these animals. In the near-term fetus and the neonate, the blood-brain barrier is apparently complete to catecholamines (Evans *et al.*, 1974), and so peripheral rather than central stimulation of ventilation might be expected. In fish, the blood–brain barrier allows the passage of catecholamines, especially noradrenalin (Peyraud-Waitzenegger *et al.*, 1980; Nekvasil and Olson, 1986), and the action of catecholamines could be due to a direct stimulation of the central respiratory pattern generator or respiratory motor neurones in the medulla (Waitzenegger, 1967).

The questions posed in this review are (a) to what extent are changes in the levels of circulating catecholamines involved in mediating changes in gill ventilation in fish, and (b) if circulating catecholamines are involved in modulating gill ventilation in fish, what is the mechanism of action?

Evidence for the involvement of circulating catecholamines in the control of gill ventilation in fish

Injections of catecholamines into the blood of fish can result in an increase in gill ventilation in the eel (*Anguilla anguilla*, Apodes) (Peyraud-Waitzenagger, 1979), the rainbow trout (*Oncorhynchus mykiss*, Salmonidae) (S. Aota, personal communication), the dogfish (Taylor and Wilson, 1989) and bowfin (*Amia calva*, Holostei) (McKenzie, 1990). In the trout and the bowfin these effects have been blocked by prior infusion of the β-adrenergic antagonist propanolol. The hyperventilatory response has been observed in summer eels and trout but in winter these fish show an α-adrenergic mediated hypoventilation in response to catecholamine infusion (Peyraud-Waitzenegger, 1979; S. Aota, personal communication).

A clear experimental demonstration of a role for increased circulating catecholamines in mediating ventilatory changes was recently provided by acid infusion into trout (Fig. 4; Aota *et al.*, 1990). Acid infusion caused a release of catecholamines into the blood (Boutilier *et al.*, 1986) which was associated with an increase in ventilation that was blocked by prior infusion of propranolol (Aota *et al.*, 1990). Exposure of the trout to hyperoxia blocked the release of catecholamines and the increase in ventilation following acid infusion. Thus an acidosis per se was not sufficient to induce a ventilatory response, which only occurred if there was a release of catecholamines consequent upon acid-induced hypoxaemia (Perry *et al.*, 1989; Aota *et al.*, 1990). It is possible, of course, that the increases in circulating catecholamines and gill ventilation were not causally related, but that the acidosis generated the increased ventilation via a pathway containing a β-adrenergic synapse and this reflex was blocked by hyperoxic conditions. The simplest explanation, however, is that acid infusion results in catecholamine release, which then stimulates ventilation. The acidosis following burst swimming is also associated with high catecholamine levels in the blood (Primmett *et al.*, 1986) and may stimulate gill ventilation during the period of the repayment of oxygen debt in the post-exercise fish. A hypoxia-induced increase in ventilation volume in the trout was abolished by injection of propranolol, providing evidence for a direct relationship between catecholamines and hypoxaemic hyperventilation.

Dogfish that are restrained or disturbed by experimental conditions show no ventilatory response to either hypoxia (Butler and Taylor, 1971) or catecholamine infusion (Taylor and Wilson, 1989), presumably because ventilation rate and circulating catechol-

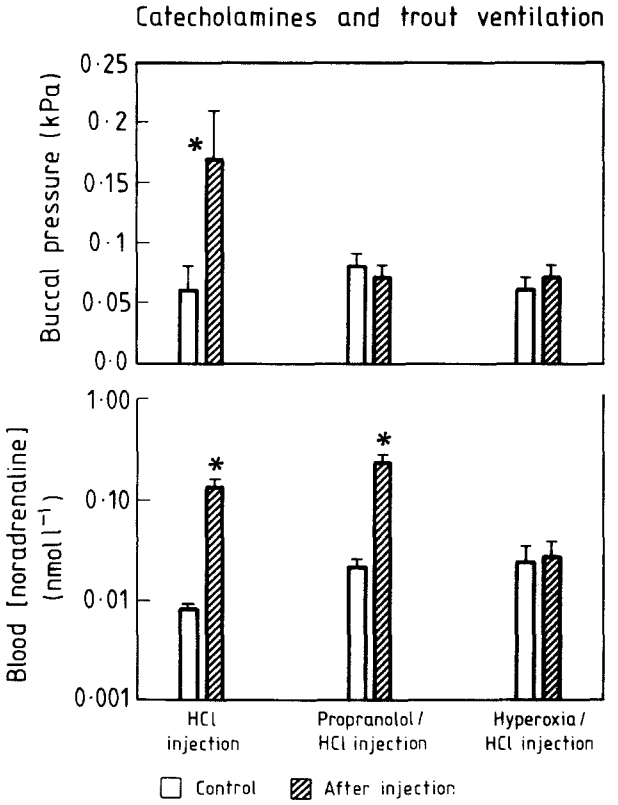


Fig. 4. Changes in ventilatory pressures and blood noradrenalin levels (mean values ± SEM) in rainbow trout injected with 0.04 mol l^{-1} HCl, injected with 2.5×10^{-4} mol l^{-1} propranolol prior to HCl injection, or exposed to hyperoxia prior to HCl injection ($n = 6$ in all three treatments; open bars, control; hatched bars, after injection; * indicates a significant increase ($P < 0.05$) following injection). Adrenalin levels were the same as noradrenalin. Acid injection caused ventilation and blood catecholamines to rise. This effect was absent in hyperoxic fish, and propranolol abolished the ventilatory response. (Redrawn from Aota *et al.*, 1990.) Note logarithmic scale for noradrenalin.

amine levels are already high and any response is saturated. However, unrestrained dogfish, breathing at a low rate, show a marked increase in ventilation in response to both hypoxia (Metcalfe and Butler, 1984) and catecholamine infusion (Fig. 5). Hypoxia causes an increase in circulating catecholamines, especially noradrenalin, in dogfish (Butler *et al.*, 1978). These fish show a clear hypoxic bradycardia reflex (Butler and Taylor, 1971; Taylor *et al.*, 1977) which is blunted by transection of cranial nerves IX and X and abolished by the subsequent transection of V and VII, indicating the existence of oxygen receptors distributed diffusely in the orobranchial and parabranchial cavities around the gills (Butler *et al.*, 1977). However, there is no evidence for or against the presence of internal oxygen chemoreceptors involved in the initiation of ventilatory responses to hypoxia. It is possible that dogfish, and perhaps elasmobranchs in general, do not possess a chemoreceptor-driven hypoxic ventilatory reflex, but that any increases in ventilation are mediated entirely by elevation of circulating catecholamines. Thus changes in circulating catecholamines may play a much more dominant role in ventilatory increases associated with hypoxia in elasmobranchs than in teleosts, where chemoreceptor

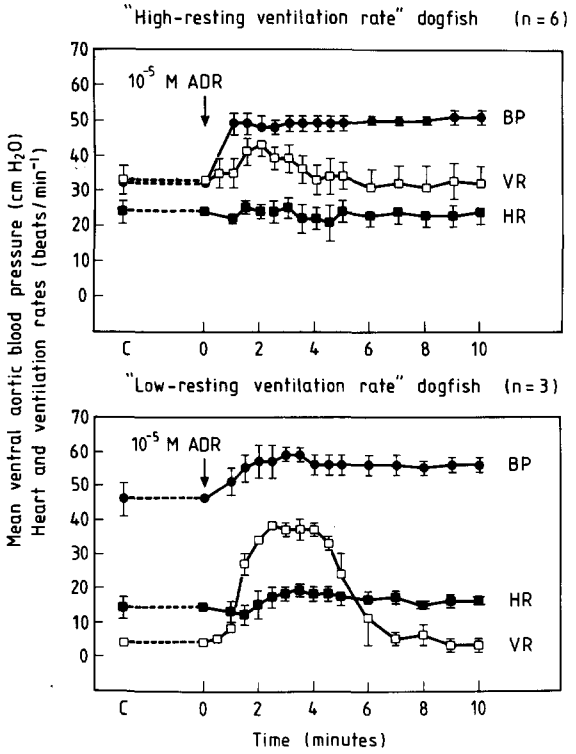


Fig. 5. Changes in blood pressure (BP), heart rate (HR) and ventilation rate (VR) in two groups of dogfish ($n = 9$) following intra-arterial injection (arrow) of adrenalin (ADR - 1 ml 10^{-5} mol l^{-1}). (Plotted values are means ± SEM.) The fish, carrying ventral aortic and orobranchial cannulae, were held unrestrained in aerated seawater. Adrenalin injection caused an increase in blood pressure in all fish, but ventilation rate increased markedly only in fish having low initial ventilation rates. C denotes the mean control values prior to adrenalin injection. (Redrawn from Taylor and Wilson, 1989.)

drive dominates in mediating ventilatory responses to hypoxia.

It seems clear that changes in circulating catecholamines have a marked effect on gill ventilation. During periods of disturbance, catecholamines are released and increase metabolism; a concomitant catecholamine stimulation of gill ventilation would maintain oxygen delivery. Thus catecholamines may play an important role in adjusting ventilation to metabolism in disturbed and post-exercise states. Catecholamines are released following burst, but not aerobic, swimming in fish. Thus catecholamines can play little or no role in increases in breathing associated with aerobic exercise but could play a major role during and after burst activity, maintaining oxygen delivery in the post-exercise acidotic state, during the period of repayment of the oxygen debt. Increases in ventilation during hypoxia in teleosts are largely mediated by stimulation of peripheral chemoreceptors, and during mild hypoxia are independent of catecholamines.

Mechanism of action of catecholamines

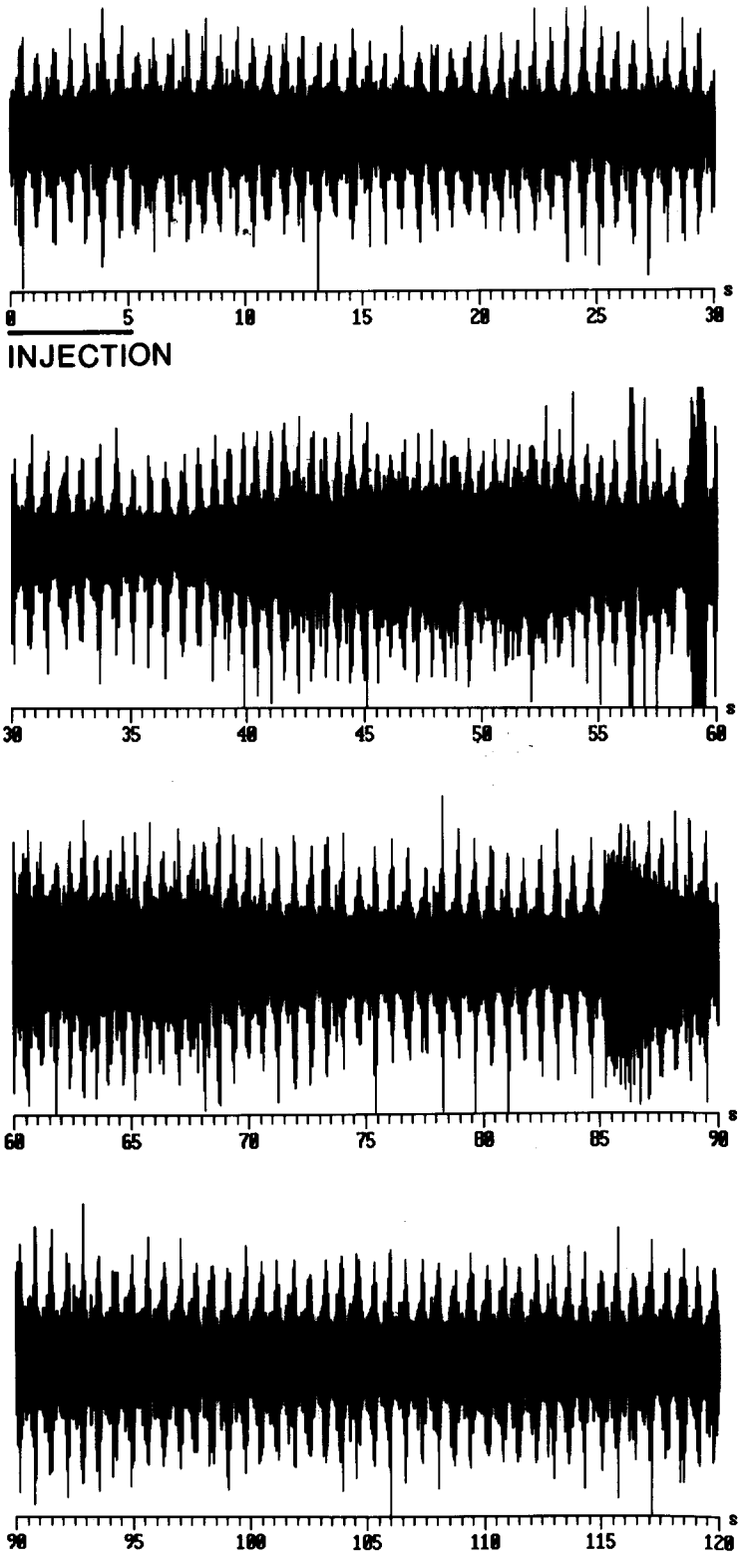
The gill oxygen chemoreceptors in trout increase discharge in response to falling oxygen levels but are insensitive to changes in circulating catecholamines (M.L. Burleson,

personal communication). Thus, both fish chemoreceptors and the mammalian carotid body respond to lowered oxygen by a process that is independent of catecholamines, although the cat, rabbit and lamb carotid body are sensitive to catecholamines by some mechanism that is independent of the process of oxygen chemoreception and transduction (Mulligan *et al.*, 1986). It seems, therefore, that catecholamines do not exert an effect on gill ventilation in fish by stimulation of peripheral chemoreceptors. Below a PO_2 of about 30 torr, however, fish chemoreceptors show a marked inhibition of activity (M.L. Burleson, personal communication). It is at about this PaO_2 that catecholamines are released into the blood of trout (Thomas *et al.*, 1988), and it is possible that in trout, increased ventilation in extreme hypoxia is maintained by increased circulating catecholamine levels, via a pathway other than the gill chemoreceptive reflex. Denervation of gill chemoreceptors in the bowfin deletes the rapid increase in gill ventilation seen in response to hypoxia in the intact animal, but a slow increase is retained, similar in time of course to that seen following catecholamine infusion in the denervated animal (McKenzie *et al.*, 1991). Thus the release of catecholamines during extreme hypoxia may maintain ventilation at high rates, compensating for the inhibition of peripheral receptors.

It is possible that the catecholamine-induced ventilatory changes are secondary responses to changes in blood pressure or blood distribution caused by increased levels of circulating catecholamines. This seems unlikely because cardiovascular effects of catecholamines can be induced at lower circulating levels, below that required to invoke ventilatory responses; that is, the cardiovascular responses can be elicited without invoking ventilatory responses, indicating separate pathways of the cardiovascular and ventilatory responses (S. Aota, personal communication). Catecholamine release may invoke changes in blood composition similar in type to that seen following acid infusion in mammals, where platelet breakdown results in thrombin release which acts as the ventilatory stimulus, rather than the acid infusion per se (Shams *et al.*, 1988). This type of response cannot as yet be completely ruled out, but whether catecholamines have a primary or secondary role, adrenergic receptors are involved in the reflex pathway.

As already stated, hyperventilation in response to catecholamine infusion or elevated levels of circulating catecholamines is inhibited by prior infusion of β-adrenergic antagonists such as propranolol, indicating that the response is mediated via β-adrenergic receptors. Conversely, hypoventilatory responses appear to be mediated via α-adrenergic receptors (Peyraud-Waitzenegger, 1979). Noradrenalin has a more potent effect on ventilation in trout and crosses the blood–brain barrier of trout more easily than adrenalin (Nekvasil and Olson, 1986), providing circumstantial evidence for a central action of noradrenalin in stimulating ventilation in trout. Recent work on dogfish has monitored central respiratory drive in the form of bursting efferent activity recorded from the central cut ends of branchial branches of the vagus nerve, which innervate respiratory muscles in the gill arches (Barrett and Taylor, 1985). Bolus infusion of catecholamines into the caudal vein of curarized and force-ventilated dogfish caused a stimulation of bursting efferent activity in branchial branches of the vagus. The onset of stimulation occurred 40–120 s after injection (Fig. 6), a lag which was consistent with the time taken for the bolus injection to reach the brain (Randall and Taylor, 1989). Often the increase in activity was repeated after a similar delay, as the bolus travelled around the circulation, returning to the brain a second time, supporting the idea of central stimulation of breathing by catecholamines. This effect was blocked by prior injection of propranolol.

When catecholamines were injected into a curarized, hyperoxic dogfish in which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activity in a hypobranchial nerve, which innervates feeding muscles, was recorded simultaneously with that in a branchial branch of the vagus, direct evidence of nervous recruitment was observed (J.J. Levings and E.W. Taylor, unpublished observations). In the hyperoxic fish prior to injection of adrenalin, the branchial branch showed regular bursting activity, but the hypobranchial nerve showed low levels of activity and only fired intermittently. Injection of adrenalin caused high levels of respiration-related, bursting activity in the hypobranchial nerve, which resembled or exceeded that observed in the disturbed normoxic and fictively hypernoeic fish. In the breathing fish this would result in recruitment of feeding muscles into forced ventilation, and this result seems to identify a role for circulating catecholamines in the onset of forced ventilation.

We are now investigating the possibility that catecholamines exert their effects directly upon the central pattern generator or the respiratory motoneurones by injection of catecholamines into the CNS. Injection of small volumes (8–20 μl) of 10^{-4} molar solution of adrenalin into the fourth ventricle of the spiny dogfish (*Squalus acanthias, Squaloidea*) caused a marked change in the pattern of central respiratory drive (measured as bursting activity in branchial nerves) (Fig. 7; Taylor and Randall, 1990; Randall, 1990). The response was complex but stereotyped. Injection was followed after about 100 s by a slowing of the rate of bursts, accompanied by a huge increase in the activity within each burst, apparently owing to the recruitment of units having larger recorded spikes, which implies larger fibre diameters because all vagal fibres are myelinated (Short *et al.*, 1977). Increased efferent activity within bursts implies that the increase in ventilation brought about by injection of catecholamines may result from an increase in stroke volume, rather than rates of contraction of the respiratory apparatus, which agrees with the observations on trout during acid infusion (Aota *et al.*, 1990). However, as the response developed, the rate and amplitude of the bursting activity increased. These responses to injected catecholamines were blocked by simultaneous injection of propranolol, which may have unmasked an α-adrenergic recepter-mediated inhibition (Fig. 7).

These data imply that areas in the CNS, accessible from the fourth ventricle, respond to an increase in catecholamine concentration with an increase or an underlying decrease in central respiratory drive. The vagal respiratory motoneurones, with efferent axons in the branchial nerves, lie in the dorsal vagal motor nucleus, which is situated bilaterally in the medulla in a medial position close to the wall of the fourth ventricle (Fig. 8: Withington-Wray *et al.*, 1986). These neurones are rhythmically active, under the influence of the central pattern generator, and supply the efferent innervation to intrinsic respiratory muscles in the gill arches. Their location close to the wall of the fourth ventricle may have caused them to be directly affected by the injection of catecholamines into this location and their subsequent diffusion into the brainstem.

Identification of the site of action of catecholamines on respiratory motor neurones in the medulla is now being investigated using microelectrode studies of single, identified neurones. Glass microelectrodes are being used to detect extracellular activity in preganglionic vagal respiratory motoneurone cell bodies in the dorsal vagal motonucleus of the

Fig. 6. Effect of intravenous injection of a bolus (1 ml 10^{-5} mol l^{-1}) of noradrenalin on efferent activity (a measure of central respiratory drive) recorded from the third branchial branch of the vagus on the left side of a dogfish (male *Scyliorhinus canicula*, 980 g). The animal was curarized and force-ventilated with hyperoxic seawater. After a delay of 40 s, bursting rate increased, culminating in a large burst of activity at 58 s, repeated at 85 s.

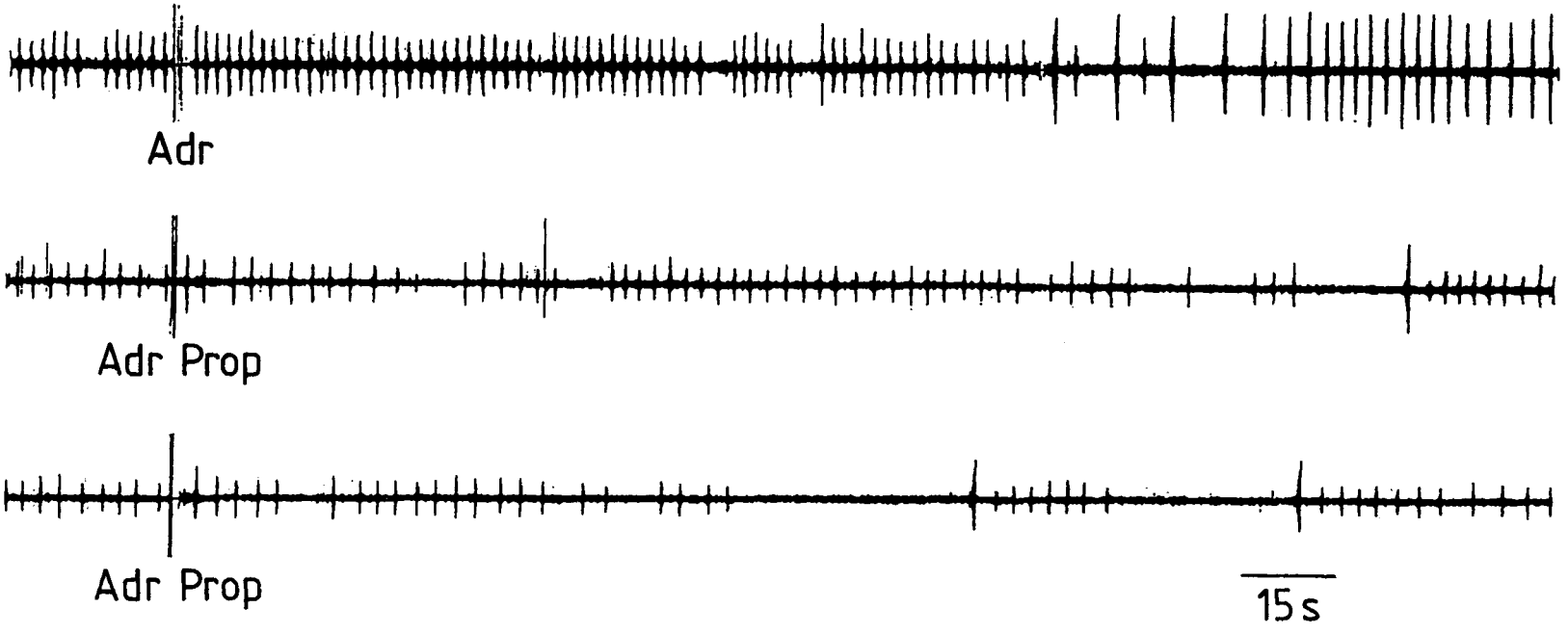

Fig. 7. Effect of injection of adrenalin into the fourth ventricle of a dogfish (male *Squalus acanthias*, 1260 g) upon bursting efferent activity recorded from the central cut end of the third branchial branch of the vagus. Injection of 20 µl of 10^{-4} mol l^{-1} adrenalin (Adr), after a delay of about 100 s, induced slow bursts of increased amplitude, which progressively increased in rate. Repeated injections of adrenalin together with propranolol (Adr Prop) abolished the stimulatory response, and on the second injection may have unmasked an α-adrenergic inhibition of efferent respiratory activity.

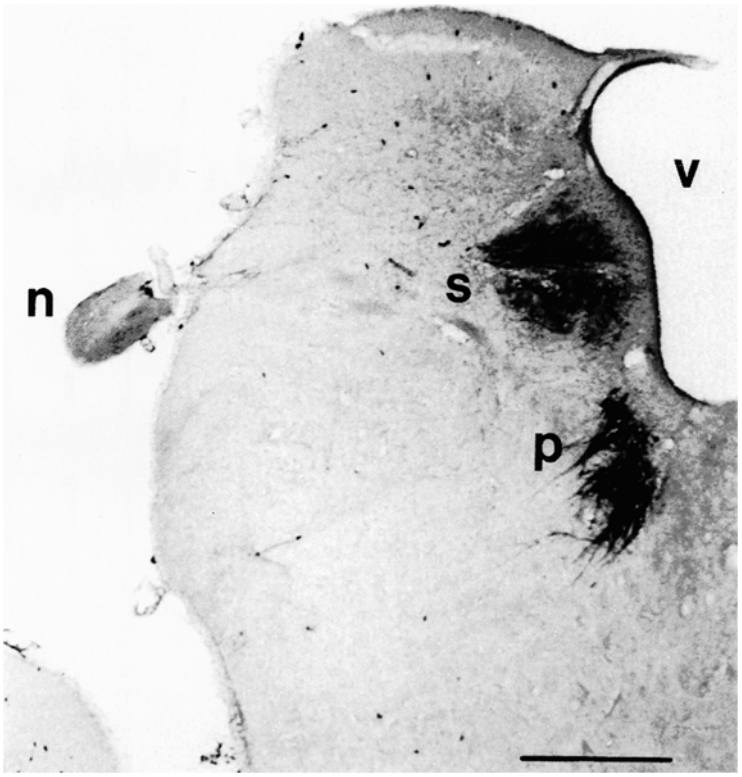

Fig. 8. Part of a 60 μm transverse section through an elasmobranch hindbrain, taken approximately 1.3 mm rostral of obex following application of horseradish peroxidase (HRP) to the third branchial branch of the vagus nerve (n is the nerve stump). Darkly stained reaction products of HRP histochemistry reveal a sensory projection (s) into the vagal sensory nucleus and preganglionic vagal respiratory neurones (p) in the dorsal vagal motonucleus close to the lateral wall of the fourth ventricle (v). Scale bar, 1 mm. (Provided by J.J. Levings.)

dogfish medulla. Whilst activity is recorded simultaneously from these nerve cell bodies and from the branchial nerves receiving their efferent axons, small volumes (< 1 μl) of 10^{-5} molar noradrenalin are injected into the recording site through a second microelectrode barrel pulled with the recording electrode.

Injection of noradrenalin close to the cell body of a respiratory neurone typically causes an increase in its firing rate which occurs within 3–5 s of the injection and is of short duration, indicating that the recording site is close to the adrenergic receptors (Fig. 9). This is in contrast to the long latency of the response to injection of catecholamines into the circulatory system (Figs 5 and 6) or in the fourth ventricle (Fig. 7), when they have to cross the blood–brain barrier in order to stimulate central sites of action. The stimulatory effect of injected catecholamines can be blocked by propranolol infusion into the blood, indicating not only that the effect is via β-adrenergic receptors but also that the site is accessible from the blood.

The short latency and duration of the stimulatory effect of injected noradrenalin on respiratory neurones indicates that they can be directly affected by local concentration.

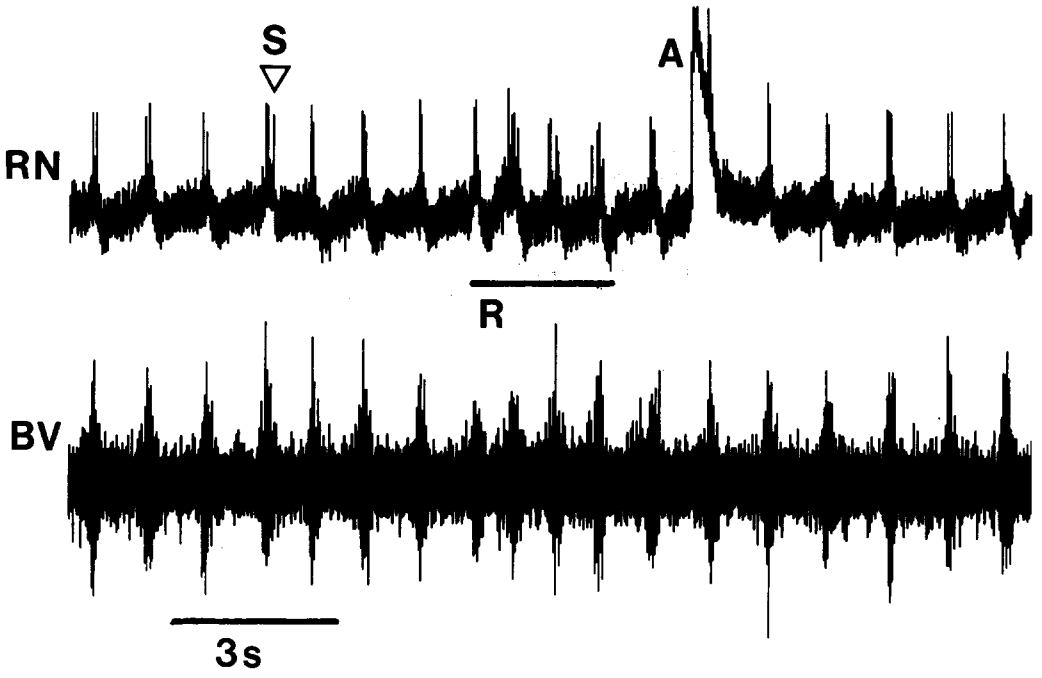

Fig. 9. Recording from a curarized and force-ventilated dogfish (female *Scyliorhinus canicula*, 840 g) of respiratory related activity from a central respiratory neurone (RN) located close to the tip of a microelectrode in the medulla about 1.5 mm rostral of obex, together with a simultaneous recording (BV) of efferent activity in the third branchial branch of the vagus nerve on the same side (left) of the fish. Concurrent bursting activity accelerated briefly following pressure injection of noradrenalin into the central recording site (S). The response (R) was of short duration (3 s), commenced 4 s after injection, and consisted of an acceleration in bursting rate (i.e. reduced inter-burst interval) and an increase in number of spikes per burst from 2–3 to 3–5. The recording of central activity includes an artefact (A); a burst of activity can be observed on its descending slope.

However, increased activity is one of many observed responses; inhibition has also been recorded on a few occasions, and some responses appeared more complex, with inhibition following stimulation on successive central injections of noradrenalin, or previously silent cells commencing to fire. Clearly, further characterization of these responses is required and work continues.

Central H^+ versus catecholamine effects

An acidosis without catecholamine release does not stimulate ventilation in fish, and hyperoxic conditions – although associated with a hypercapnic acidosis – cause hypoventilation, not hyperventilation. A correlation between pHa (pH of arterial blood) and ventilation has been reported both in teleosts (Janssen and Randall, 1975) and in elasmobranchs (Randall, 1974; Iwama, 1986) but no direct effects of changes in pHA on ventilation have been demonstrated. The only direct evidence for a central H^+ response is that mentioned by Shelton (1970), who injected small quantities of saline containing carbon dioxide into the medulla of the tench, (*Tinca tinca*, Cyprinidae) and recorded changes in breathing move-

ments. Thus, although it is often presumed to exist, there is little evidence to support the presence of a central H^+ receptor in fish.

In the aquatic environment, because carbon dioxide is much more soluble in water than oxygen, respiration is regulated primarily with reference to oxygen supply rather than CO_2 excretion, and a central H^+ receptor is not required (Randall, 1990; Randall and Cameron, 1973). Thus, it is possible that the control system for regulating gill ventilation consists of peripheral oxygen chemoreceptors and a central catecholamine-sensitive system, the former to adjust ventilation to oxygen availability during normoxia and moderate hypoxia, the latter to adjust ventilation to metabolism when oxygen supply is limiting. In terrestrial vertebrates, where ventilation is closely coupled to carbon dioxide transfer and the regulation of pHa, the central H^+ receptor system dominates over the actions of catecholamines. The development of a tight blood–brain barrier in adult mammals reduces any central action of elevated circulating catecholamines, but a peripheral action may have evolved, retaining a role for catecholamines in matching ventilation to metabolic requirements. This occurs because carotid body chemoreceptors are sensitive to changing levels of circulating catecholamines (Milsom and Sadig, 1983). These differences in ventilatory control between aquatic and terrestrial mammals are, to some degree, reflected in mammalian development; that is ontogeny recapitulates phylogeny. Fetal mammals, like fish, have a more leaky blood-brain barrier and a reduced central CO_2 drive, compared with adult mammals, and it is possible that central stimulation of breathing via changing circulating catecholamine levels is important in fetal mammals. Thus, the transition from water breathing, dominated by oxygen transfer requirements, to air breathing, dominated by carbon dioxide transfer requirements and regulation of pHa, involves a change in control systems. Aquatic breathing is regulated via peripheral oxygen chemoreceptors and central catecholamine reflexes; air breathing is dominated by central CO_2/H^+ responses plus peripheral chemoreceptor reflexes, modulated by circulating catecholamine levels.

Summary

Our current knowledge of the control of ventilation in fish is incomplete at all levels. The respiratory rhythm originates in a medullary central pattern generator (CPG), which has yet to be clearly identified and characterized. Its activity is directly modulated by inputs from elsewhere in the CNS and from peripheral mechanoreceptors. The central location of respiratory motoneurones, innervating the various respiratory muscles, has been described in detail for some fish, particularly elasmobranchs. We are still unclear, however, about the link between the CPG and the sequential firing of the motoneurones, which result in coordinated contractions of the respiratory muscles, and about the mechanisms that result in recruitment of feeding muscles into forced ventilation. In teleosts, ventilation is matched to oxygen requirements by stimulation of gill chemoreceptors, which seem to respond to oxygen content or supply. There is little evidence of a role for these receptors in elasmobranchs.

Chemoreceptor stimulation evokes a number of reflex changes in the respiratory and cardiovascular systems of fish that are rapid in onset and seem adaptive (e.g. increased ventilation and a bradycardia in response to hypoxia). Conditions that result in hypoxaemia and the consequent ventilatory changes also cause an elevation in circulating catecholamine levels. We have explored the possibility of a causal relationship between these

levels and the ventilatory response. Strong evidence for this relationship arises from experiments on hypoxia and acid infusion, which trigger a ventilatory increase and a rise in circulating catecholamines. Both ventilatory responses are blocked by an injection of propranolol, indicating that β adrenoreceptors are involved in the response.

The ventilatory response to hypoxia, in teleosts at least, occurs very rapidly, perhaps before any marked increase in circulating catecholamines and almost certainly before any blood-borne catecholamines could reach the respiratory neurones. This argues for an immediate neuronal reflex based on chemoreceptors in the gill region responding to hypoxia. Clearly, circulating catecholamines also affect ventilation through some action in the medulla and could act in concert with a direct neuronal chemoreceptive drive during hypoxia. The studies on acid infusion during hyperoxia, where there is an acidosis but no increase in ventilation or blood catecholamines, would argue against any hydrogen ion receptor, either peripheral or central, being involved in the reflex ventilatory response to acidotic conditions in fish.

The release of catecholamines into the circulation, therefore, seems to be an absolute requirement for the ventilatory response to acidosis in fish. Present evidence supports a role for β-adrenergic receptors on respiratory neurones, stimulated by changes in the levels of circulating catecholamines, in the control of ventilatory responses to marked changes in oxygen availability in fish, such as those occurring in the post-exercise acidotic state.

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