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THE CONTROL OF VENTILATORY AND CARDIAC RESPONSES TO CHANGES IN AMBIENT OXYGEN TENSION AND OXYGEN DEMAND IN OCTOPUS

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Summary

Octopus vulgaris **can regulate its oxygen uptake down to a P_{O_2} of around 6.7 kPa. As the tension falls from 18.6 to 6.7 kPa (140 to 50 mmHg), *P*V (the pressure pulse driving the ventilatory flow, measured inside the mantle cavity) can more than double while *f*V (the ventilation frequency) increases by a few per cent at most. Both changes are reversed when the ambient oxygen tension is returned to normal. Cutting the visceral nerves linking the hearts and gills to the brain prevents these adaptive changes in *P*V and *f*V, as does section of the branchial nerves linking the cardiac ganglia to the gills. Responses to changes in ambient oxygen tension are very fast, beginning within two or three ventilation cycles. It is concluded that changes to *P*V and *f*V depend upon receptors in the gills and on the integrity of a nervous pathway to the brain.**

Changes in oxygen tension also affect the hearts, where aortic pulse amplitude (*P*a) and, to a lesser extent, heartbeat frequency (f_H) fall and rise with the ambient P_{O_2}. In this case, section of the visceral or branchial nerves has no effect. Responses are again very rapid. It is concluded that the observed fall and return to normoxic values of *P*a and f_H are local responses to a fall and rise in the oxygen tension of blood coming from the gills into the systemic heart.

Changes to ventilation and heartbeat can also occur in normoxic water when oxygen demand rises after feeding. These responses are not prevented by section of the visceral or branchial nerves. Possible control of ventilation and heartbeat through the neurosecretory system in the anterior vena cava is discussed.

Key words: ventilation, heartbeat, oxygen, *Octopus vulgaris.*

Introduction

Any study of ventilation in cephalopods is complicated by the fact that the wall of the mantle cavity has a wide range of functions besides the generation of a flow of water through the gills. It is used to create the jet that is, in many cases, responsible for locomotion. It encloses the anus and the kidney ducts and must periodically assist in the elimination of the wastes that these discharge. It encloses the reproductive openings and plays a part in passing eggs or spermatophores to the outside (Wells and Wells, 1972). It evidently houses chemoreceptors, because the animals react with ventilatory changes to a range of stimuli (extracts from their crustacean prey for example) released into the inhalant respiratory stream (Boyle, 1983; Chase and Wells, 1986).

Octopuses, even at rest, 'cough' from time to time, presumably to expel irritants or to discharge wastes from the anus or renal sacs, and on these occasions there are a large transient increases in *P*V and *f*V.

When an octopus is fed or moves about, its oxygen uptake rises. At the same time, ventilatory movements increase in magnitude. In the case of feeding, this could be due, in part, to the need to discharge wastes or to arousal caused by chemical stimuli in the water. If the animal takes exercise, the same responses occur but, in this case, the interpretation of the changes is confused by the fact that the animal periodically uses its jet to assist locomotion.

A less complicated situation is found in the response to changes in ambient oxygen partial pressure. Here, ventilatory changes will be related to gas exchange only, provided that the animal remains undisturbed and does not become spontaneously active.

At rest, in normoxic water, *Octopus vulgaris* can extract as much as 65 % of the available oxygen from the ventilation stream. This proportion is not increased significantly as the P_{O_2} falls (Wells and Wells, 1982, 1985).

The animal can maintain its rate of oxygen uptake as the ambient P_{O_2} falls to around 6.7 kPa; for some individuals, the rate of uptake remains almost constant down to 3.5 kPa (Wells and Wells, 1983). The individual level is presumably related to the very variable oxygen-carrying capacity of the blood, which can range from 2 to 5 vols% (M. J. Wells, unpublished data from 37 octopuses).

Taken together, the observations on oxygen extraction and

the capacity to maintain oxygen uptake imply that the animal must be able at least to double, and sometimes to increase fourfold, its ventilatory throughput as P_{O_2} falls in the incoming stream.

Ventilation stroke volumes have been calculated from the inhalant and exhalant P_{O_2} and the animals' oxygen uptake (Wells and Wells, 1985). These (Fick principle) calculations indicate increases in stroke volume (*V*S) of up to 400 % (mean ± S.E.M. from 10 animals, 161±39 %), as the ambient P_{O_2} fell from 18.6 to 6.2 kPa. Ventilation frequency (*f*V), observed directly, increased by only 32±4 %. Similar calculated increases in *V*S of around 300 % were found in exercising octopuses by Houlihan *et al.* (1986), again with little change to *f*V.

It should be noted in passing that the large changes in *V*S found in *Octopus* are not necessarily typical of cephalopods in general. In *Lolliguncula brevis*, for example, both *f*V and *V*S remain almost constant during progressive hypoxia because oxygen extraction, initially low at 4–5 %, can more than double (Wells *et al.* 1988); a cephalopod that depends on continuous jet propulsion cannot afford large fluctuations in *f*V or *V*S unrelated to locomotion.

Materials and methods

Animals

Octopus vulgaris Cuvier was used in experiments carried out at the Laboratoire Arago, Banyuls, France, or the Station de Recherches Sous-Marines et Océanographiques, Calvi, Corsica. The animals were caught by trawl or SCUBA divers and held in individual tanks (generally plastic buckets with snap-on lids) until it was clear that they were undamaged and feeding normally. Animals from a wide range of sizes (23–1350 g, most between 500 and 1000 g) were used. The larger animals were nearly all males. Temperatures ranged from 19 °C (respirometry in Banyuls, end of September) to 25 °C (experiments with cyanide at STARESO, end of July). During any one experiment, the temperature rarely changed by as much as 1 °C.

Measurement of ventilation pressure and frequency

It is difficult and perhaps impossible to measure the volume of the ventilation stream in *Octopus* directly, because the pressures driving the flow are very low, of the order of 150 Pa when the animal is at rest (Wells and Smith, 1985), and any attempt to collect the output must involve touching the funnel, which is very sensitive. Winterstein (1925) measured the ventilation volume directly using the finger of a rubber glove stitched to the funnel, but his minimum and maximum reported values (100 and 1500 ml min^{-1} respectively), fall substantially below those (272 and 4011 ml min^{-1}, Wells and Wells, 1985) estimated for animals of comparable size from oxygen extraction and uptake rates.

For most of the experiments on ventilation to be described below, ventilatory frequency (*f*V) and pulse pressure (*P*V) were measured through a 1 mm (o.d, 0.75 mm i.d) nylon cannula terminating in a larger-diameter T-piece sewn to the roof of the dorsal, prebranchial part of the mantle cavity. The cannula led to an EM750 pressure transducer attached to the side of the respirometer. Data were recorded using a Goerz Servogor 110 recorder. The cannula did not appear to impede the ventilation or to restrict the free movement of the animal about its respirometer in any way. Preliminary tests showed that the calibre of the cannula was sufficient to allow accurate relaying of pressures at the rates of change occurring in ventilation, showing pulse patterns very similar to those recorded by Wells and Smith (1985) using Portex cannulae of 3–4 mm o.d. For the experiments described under *The speed of response*, below, a modified Cambridge type 72125 cardiograph was used in place of the Goerz Servogor recorder.

*P*V rises as the ambient P_{O_2} falls; percentage increases appear to mirror the calculated increases in ventilation volume. Changes in *P*V are assumed, in the absence of gross changes to *f*V, to indicate changes in ventilation stroke volume. No attempt was made to establish the exact relationship between *P*V and *V*S.

Blood pressure (*P*a) and heartbeat frequency (*f*H) were measured through a T-piece inserted into the dorsal aorta, as described in Wells (1979). The cannula used to link the aortic T-piece to the Elcomatic pressure transducer was 1.5 mm i.d. Portex (blood amoebocytes are liable to clog very fine cannulae). Again, the cannula did not appear to impede the movements of the octopus about its tank.

Cannulae were installed and all other surgical operations were carried out under 2.5 % ethanol anaesthesia (in Calvi we were obliged to use a local brandy instead, since the laboratory had no reagent-grade alcohol). Anaesthesia was very light, so that ventilation scarcely stopped, for all procedures except aortic cannulations where a deeper anaesthesia was necessary. A minimum recovery time of 1 h (2 h for aortic cannulations) was allowed between the return of regular ventilation and the beginning of any experiment in the respirometer.

Respirometry

Experiments were carried out in an opaque white plastic bucket, identical to those used to house the animals before and between experiments. The oxygen uptake of octopuses increases, and may take many hours to settle down, if the animals are placed in unfamiliar surroundings (Wells *et al.* 1983*a*). Holes in the side of the bucket allowed the water from the circulation to overflow, giving a standard volume of 19 l; in some experiments, with the smaller animals, the volume was reduced to 12–14 l when the circulation was closed, by siphoning off some of the water. The surface of the water in the bucket was open to the air. Although no attempt was made to quantify $\dot{V}_{O_2}$, the rates at which individuals reduced the oxygen level in the respirometer before and after operations could be compared.

Oxygen levels were measured using an EIL 7130 dissolved oxygen meter. Flow rate across the probe in a Perspex cell outside the respirometer was maintained by a Radiospares 330-828 peristaltic pump, taking water from and returning it to the

tank at 70 ml min^{-1}. Care was taken to ensure that the cannula sampling the water in the respirometer was always distant from the exhalant funnel of the octopus. In some of the experiments (see *The speed of response* below), a Neocath 1000 dissolved oxygen meter was used in place of the EIL 7130.

When the external circulation was removed to allow an animal to reduce the ambient oxygen level, mixing in the respirometer was maintained by the peristaltic pump and, more importantly, by the animal itself. A 1 kg octopus, at rest in normoxic water, will draw water into its mantle and expel it through the funnel at a rate of 0.5–1 l min^{-1}. This rises to 2–3 l min^{-1} as the P_{O_2} falls to 6.2 kPa (Wells and Wells, 1985). Observations of fine particles in the water suggested that even the lower ventilation rate was sufficient to keep the water in the 19 l respirometer well stirred.

Records of PV, fV, Pa and fH were made at every 5 or 10 % change in oxygen saturation. In Figs 2, 3, 4, 7 and 8 records made at 95 % and 90 %, 85 % and 80 % and so on have been pooled, so that the standard errors shown normally arise from the records of two observations from each of the animals concerned. Occasionally a recording was missed because an animal was moving as the saturation reached and passed the desired level; the standard errror is then based on a smaller number of observations. In some cases, where breathing appeared to be laboured or the animal became restive, circulation was restored at 40 % oxygen saturation, so that fewer animals were tested at 35–30 % oxygen saturation than at other times during the experiment. Where this has occurred it is noted in the captions to the figures.

For the experiments described below under *Long-term effects of visceral and branchial nerve section and the response to increased oxygen demand caused by feeding*, small (23–61 g) octopuses were housed in 2.2 l closed respirometers. There was a through-flow of well-aerated water except during periods when the oxygen uptake was being measured. On these occasions, the circulation was closed and the water recycled past the EIL 7130 oxygen electrode by a peristaltic pump for the duration of the experiment.

Anatomy of the nerve supply to the hearts and gills

The anatomy of the nervous system interconnecting the hearts, gills and brain is outlined in Fig. 1. For some of the experiments, the visceral nerves were cut at the level indicated, on the central side of the fusiform ganglia. In others, the branchial nerves were cut between the cardiac ganglia and the gills (Fig. 1). The nerves lie superficial to the viscera and are clearly visible. In each nerve-cutting operation, a few millimetres of nerve was removed, so that there was no possibility of reconnection. 24 h before the control runs preceding the nerve-cutting operations, the vertical longitudinal muscle (the ‘septum’) linking the viscera to the floor of the mantle was cut in a preliminary operation, making it possible to fold the mantle inside-out during subsequent nerve-cutting operations.

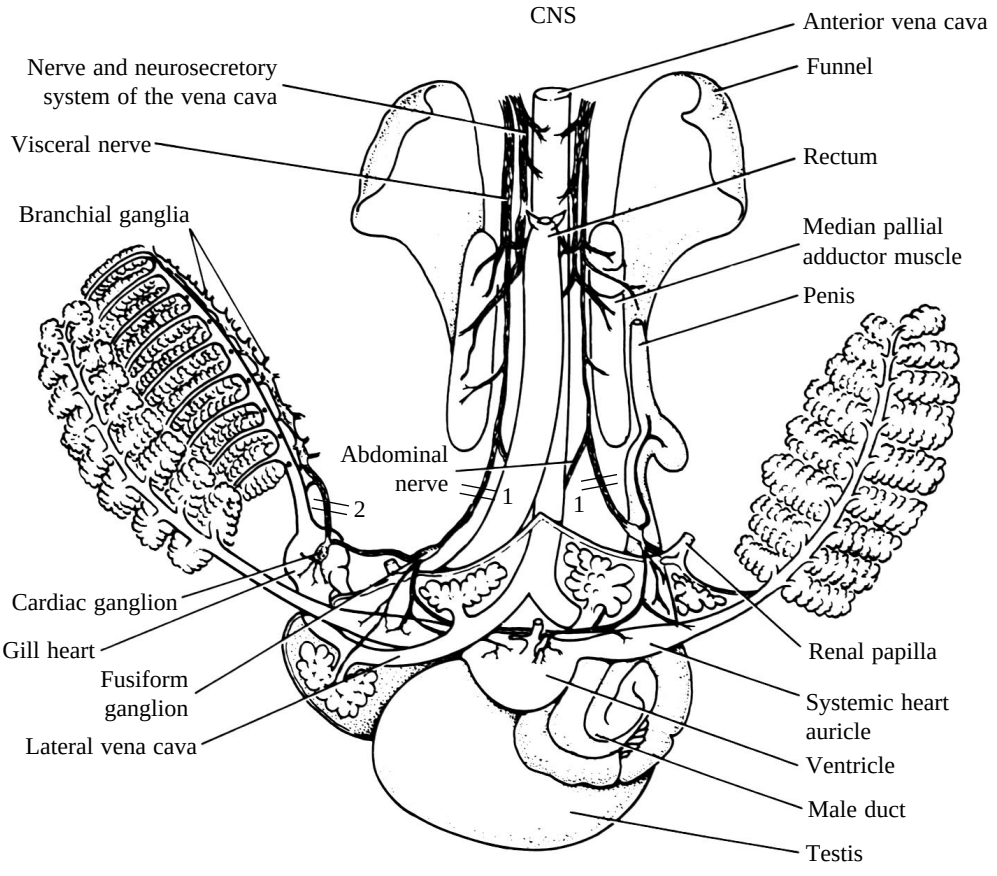

Fig. 1. Ventral view of the nerve supply to the hearts and gills, from inside the mantle cavity. The cardiac ganglion and the branchial nerve connecting this to the branchial ganglia are shown on the right side only. 1 shows where the visceral nerves were cut and 2 where the branchial nerves were cut in operations described in the text (after Young, 1967).

The nerve supply to the mantle musculature passes directly from the central nevous system to the stellate ganglia, from which nerves radiate to all parts of the musculature (Young, 1971).

Results

The ventilatory response to acute progressive hypoxia and a return to normoxia

Fig. 2A shows how PV and fV increased and decreased as five animals reduced the oxygen content in the respirometer and then responded when the circulation of normoxic water was restored. The duration of the experiment varied somewhat with the size of the individual concerned, the first phase, progressive hypoxia, lasting from 50 to 120 min, the second phase, following restoration of the circulation, 10–25 min.

PV increased threefold as the ambient P_{O_2} fell from 18.6 kPa (90 % saturated) to 6.2 kPa (30 % saturated). fV changed relatively little, rising from 0.4 to 0.5 Hz. There was a rapid return to normoxic values, tracking the rise in ambient oxygen levels following the restoration of the circulation. Similar results for control animals are shown in Figs 3A and 4A.

The effect of cutting the visceral nerves

The five animals were allowed 1–3 h to recover in normoxic water before an operation in which the visceral nerves were cut on both sides at the level of the rectum, between the fusiform ganglia and the central nervous system (see Fig. 1). After a further 1–3 h, the progressive hypoxia and recovery experiment was repeated as before (Fig. 2B). PV and fV did not differ from controls in normoxic water, but the response to a decline in ambient oxygen levels disappeared. The very large peak and S.E.M. at 30 % saturation arose because only three of the five octopuses were tested at this extreme level; one of the three produced very high (up to 304 Pa) ventilatory pulses for unknown reasons.

Two additional results of cutting the visceral nerves were noted. It generally took longer to revive the animals after anaesthesia. Whereas controls (cut the septal muscle) began to ventilate regularly within 2–3 min of the beginning of post-operational irrigation of the gills, octopuses with the visceral nerves cut often required 5 or 10 min; ventilation might begin and then stop one or more times before a regular pulse became established. There was also a marked decline in the rate of oxygen uptake. In the initial, progressive hypoxia, phase of the experiment, the five post-operational animals averaged 75 % longer to reduce the P_{O_2} from 18.6 kPa to 6.7 kPa. Because the respirometer was open, not closed, we do not know the exact rate of oxygen uptake of the animals, but the long duration of the experiments following visceral nerve section implies that the animals were no longer maintaining their previous rate of oxygen uptake.

Two of the operated octopuses were re-tested at 5 and 7 days after operation (Fig. 2C). The failure to respond to a fall in ambient oxygen tension (Fig. 2B) was not due to immediate post-operational stress; the response had not returned a week later. In this case, no tests were made at 30 % saturation.

It is concluded that the central nervous system must receive

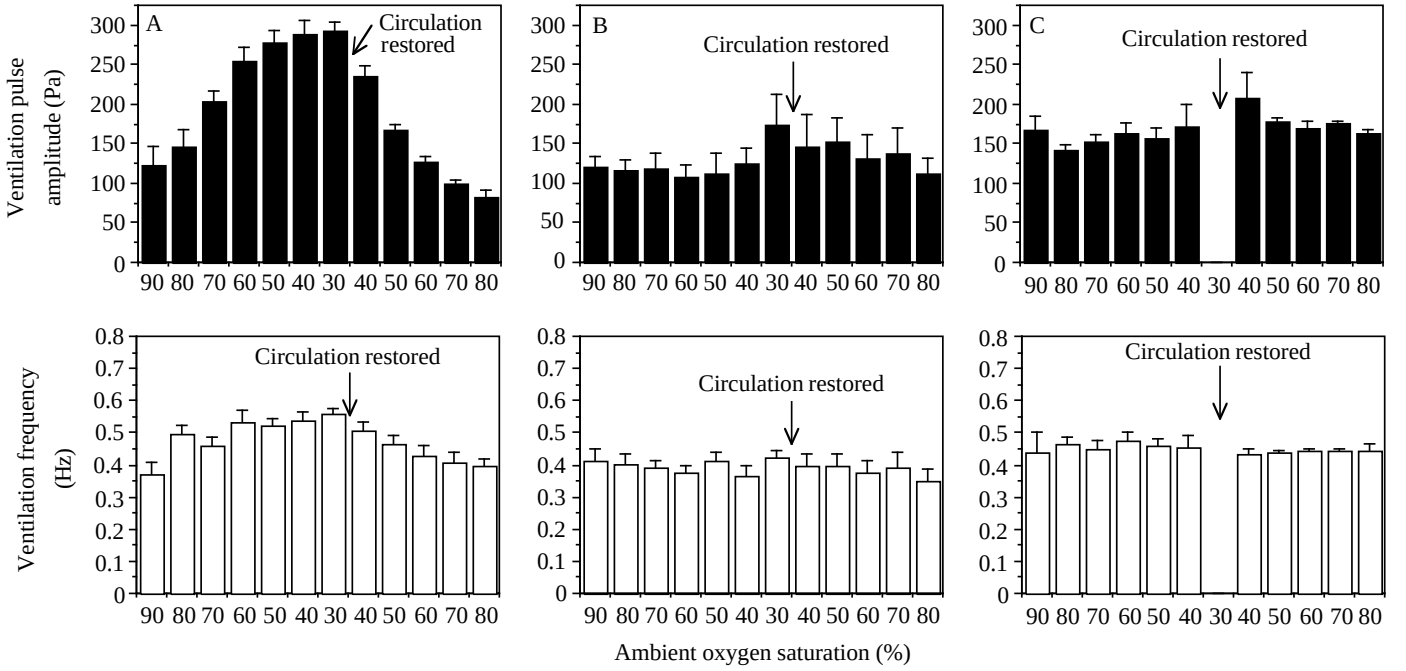

Fig. 2. Changes in ventilation pulse amplitude and frequency during progressive hypoxia (water circulation stopped) and recovery and the effect of cutting the visceral nerves. (A) Changes before and (B) after section of the visceral nerves. N=5 (in B, N=3 at 30 % oxygen saturation). (C) The same experiment repeated with two of the five animals 5 and 7 days later (no tests made at 30 % saturation). Values are mean and S.E.M.

a signal indicating the ambient oxygen tension through the visceral nerves in order to generate an adaptive output to the mantle musculature *via* the stellate nerves.

The effect of cutting the branchial nerves

Four animals were tested before and after section of the branchial nerves between the cardiac ganglia and the gills on both sides (see Fig. 1). The results (Fig. 3A,B) resemble the results of cutting the visceral nerves. The ventilatory response to changes in P_{O_2} fails (or is very considerably reduced) following the operation. Three of the animals were re-tested 2, 4 and 13 days later; again, the response did not return with time (Fig. 3C).

Two further animals had first one and then both branchial nerves cut. Fig. 4A shows the response of these animals before either operation, Fig. 4B after one nerve was cut and Fig. 4C after section of both nerves. The unilateral operation appeared to reduce the response; the second operation stopped it.

It is concluded that the receptors indicating ambient oxygen tension must be located in the gills.

The speed of response

If an octopus is allowed to reduce the oxygen tension in a respirometer beyond the point where it can still maintain its rate of oxygen uptake, *P*V and *f*V fall. By the time a P_{O_2} of 3.5 kPa is reached, both are considerably reduced below the maxima found at around 6.7 kPa in most animals. If the circulation is then restored, there is a rapid increase in *P*V and *f*V; once again the changes in *P*V are much greater than the changes in *f*V. In three animals, records of the ventilatory pulse were obtained over the period covering the restoration of the circulation. Care was taken that the circulation stream input was close to the ventilation inlet on one side of the mantle. Five such records were obtained from the three animals, with initial P_{O_2} values of 1.7–3.5 kPa. In every case *P*V was already visibly greater at the first or second ventilation cycle following restoration of the circulation. Fig. 5 shows the pulse-by-pulse change at the first run with each of the three animals.

These responses are repeatable and reversible if animals are held at low P_{O_2}. Normoxic water excites an immediate ventilatory response, which stops as the supply of well-aerated water is withdrawn. For these experiments, samples were injected through a cannula held close to the inhalant (lateral dorsal) opening of the mantle on one side. The flow rate was 2–3 ml s^{-1} for approximately 30 s. Twenty-seven such tests were made with three animals; normoxic water produced increases in *P*V within 1–3 ventilation cycles in 24 instances. There was a change in *P*V in only one out of 22 control runs, using water from the respirometer. Fig. 6 shows a sample set of responses with one animal.

Animals held at high ambient P_{O_2} and presented with low-P_{O_2} water in the same manner also showed responses, but the effect was less marked and less reliable; apparent positive responses (in this case also, increases in *P*V and *f*V, the two always seem to occur together) were found on only 10 out of 24 occasions. There were no negative responses. Control runs, using well-aerated sea water from the animal's tank, yielded one slight increase in *P*V in 22 trials. Again the

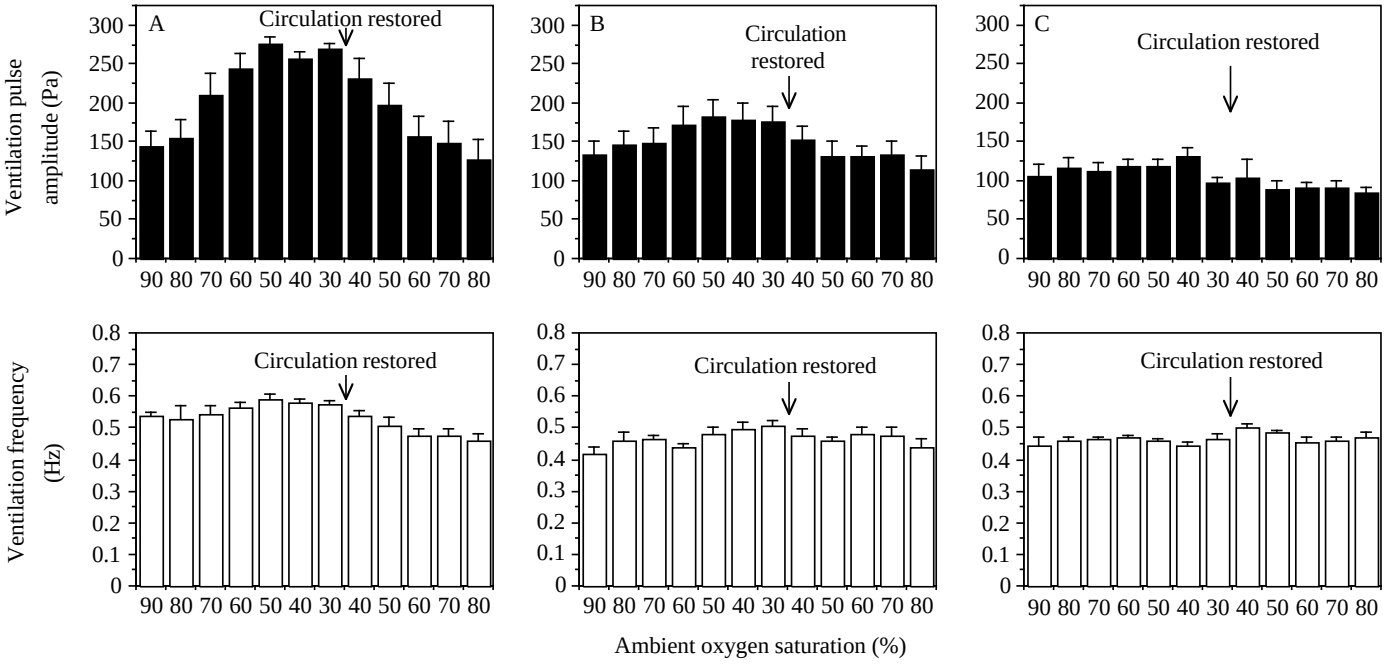

Fig. 3. Changes in ventilation pulse amplitude and frequency during progressive hypoxia and recovery and the effect of sectioning the branchial nerves. (A) Changes before and (B) after section of the branchial nerves. *N*=4. (C) The same experiment repeated with three of the animals 2, 4 and 13 days later. Other details as in Fig. 2.

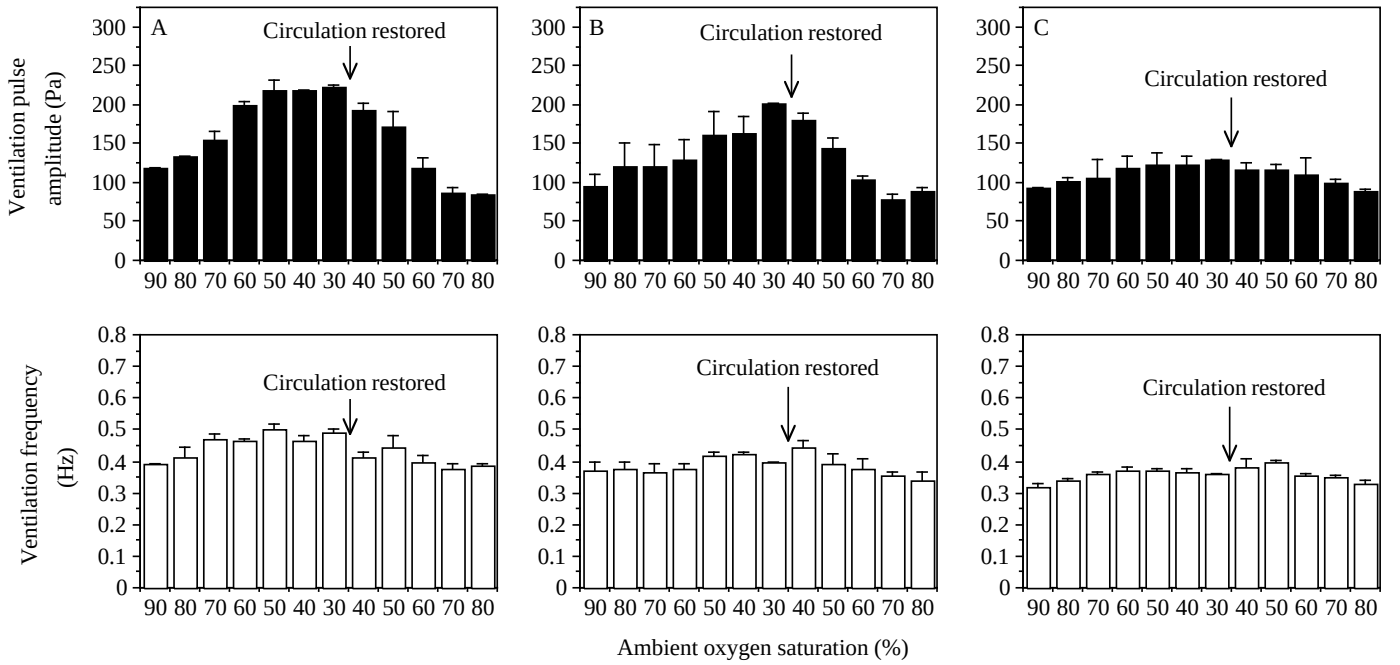

Fig. 4. The effect of cutting first one and then both branchial nerves on the ventilatory response to a fall and rise in ambient oxygen saturation. *N*=2. (A) Responses with both nerves intact, (B) responses after section of the branchial nerve on one side only and (C) after section of the second branchial nerve. Other details as in Fig. 2.

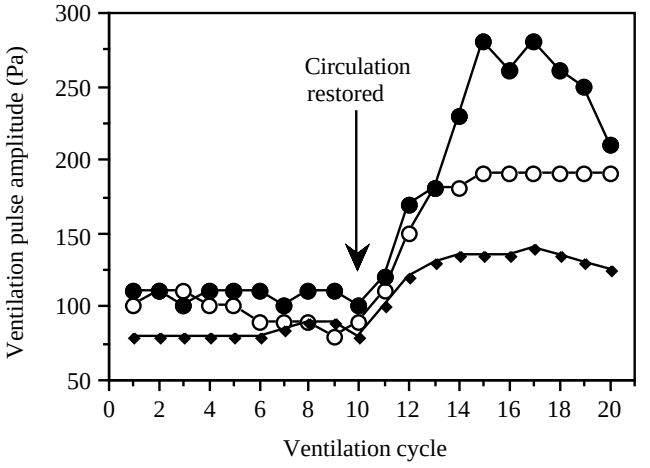

Fig. 5. Pulse-by-pulse changes in ventilation pulse amplitude in three animals immediately before and after the restoration of a circulation introducing normoxic water following a period at a very low (1.7–3.5 kPa) ambient P_{O_2}.

responses, when they occurred, took place within 1–3 ventilation cycles.

The cardiac response to acute progressive hypoxia and a return to normoxia

The response of the systemic heart to progressive hypoxia has already been described (Wells, 1979; Wells and Wells, 1983) and is opposite to the ventilatory response. As the ambient oxygen tension falls, the aortic pulse reduces in amplitude and the heart slows.

The effect of cutting the visceral nerves

Fig. 7A,B summarises the changes in heartbeat (aortic pulse) amplitude (*P*a) and heartbeat frequency (f_H) in three octopuses tested before and after section of the visceral nerves between the fusiform ganglia and the brain (see Fig. 1). A fourth animal (Fig. 7C) was not tested before visceral nerve section, but was tested twice afterwards at an interval similar to that separating the control and post-section tests shown in Fig. 7A,B; Fig. 7C shows average values from the two experiments. Cutting the visceral nerves did not appear to change the responses to a fall and rise in ambient oxygen tension.

The effect of cutting the branchial nerves

Two animals were tested before and after bilateral section of the branchial nerves at the level shown in Fig. 1 and a third octopus was tested twice after section of the branchial nerves (Fig. 8).

Apart from a small decrease in *P*a shown after the operation, there was little indication of any change in response. The third animal, tested only after the operation, had a lower *P*a and a higher f_H (so that cardiac output may have stayed the same) than the others, but again there was a fall and rise in the pulse with ambient oxygen saturation.

It is concluded that, unlike the ventilatory response, the heart's reaction to a fall and rise in ambient oxygen tension does not depend on an input from oxygen receptors in the gills.

The speed of the cardiac response

Wells (1979) and Wells and Wells (1983) reported a series

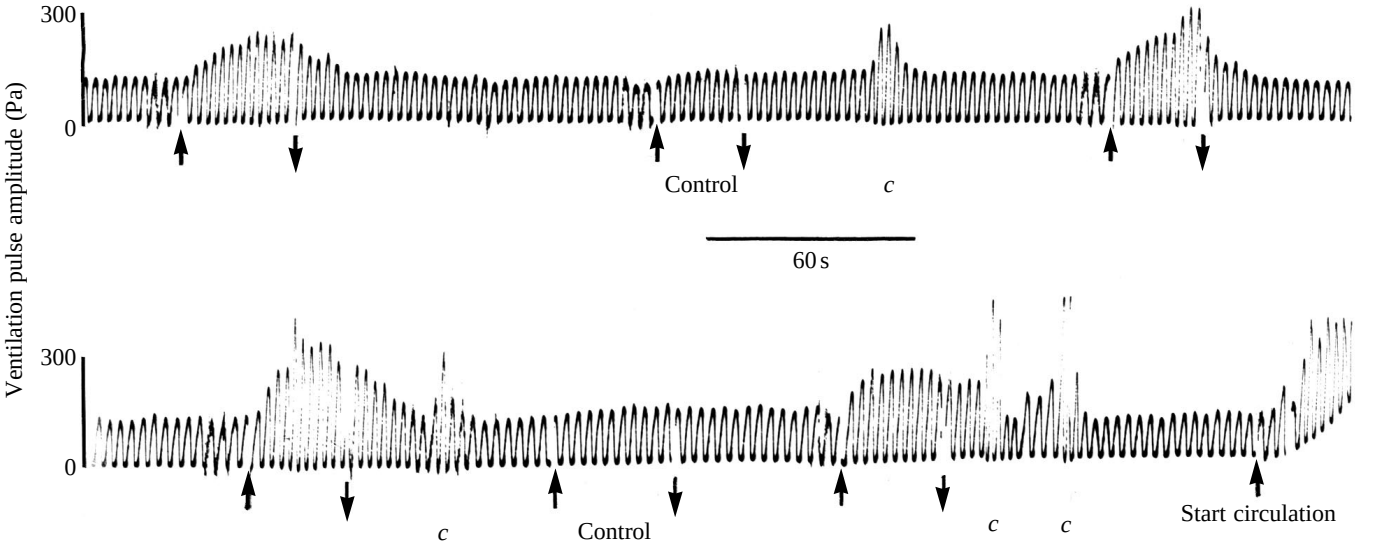

Fig. 6. The effect on ventilation pulse amplitude and frequency of injections of normoxic water released close to the inhalant opening of the mantle on one side of an octopus held at a P_{O_2} of 2.8 kPa. Control runs were made with water taken from the animal's tank. Arrows show the beginning and end of the injection of the sample in each case. *c*, Animal 'coughed'. At the end of the record, the circulation of well-aerated water was restored, raising the oxygen level in the respirometer; there was an immediate ventilatory response to the change in the oxygen content of the water.

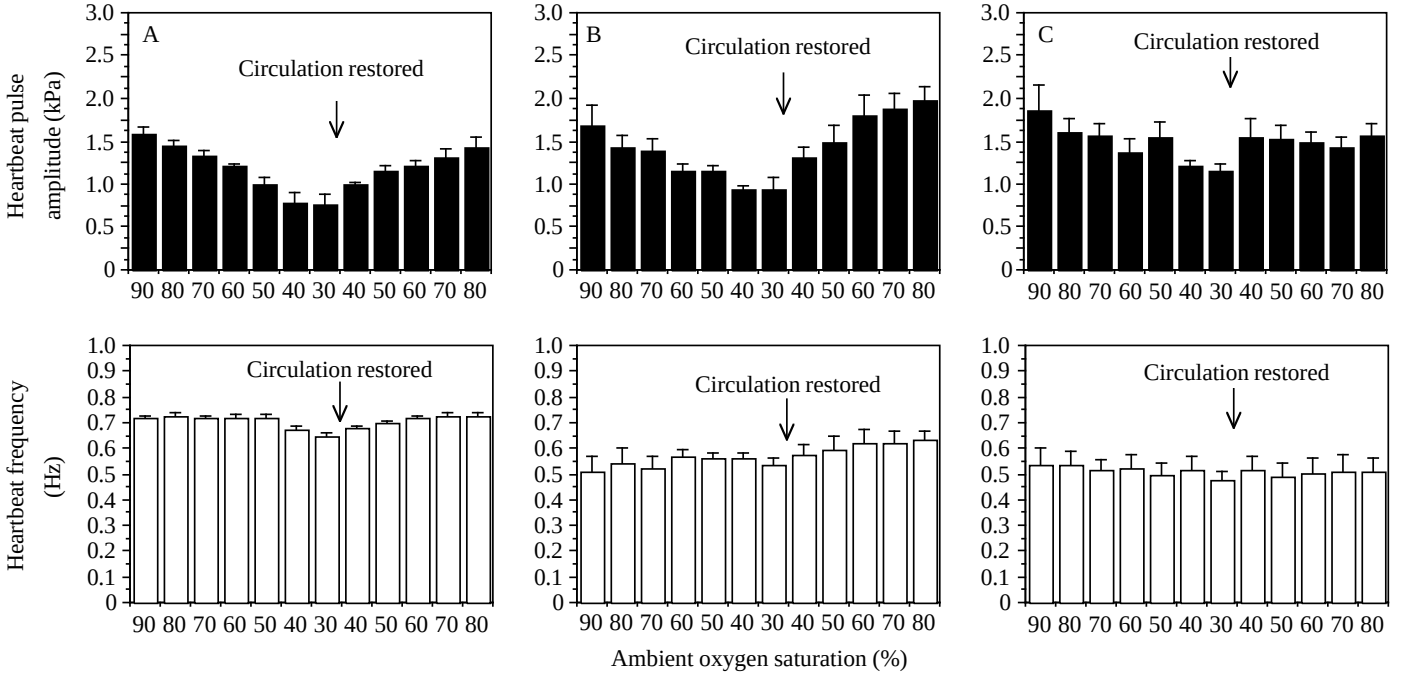

Fig. 7. The response of the systemic heart to a fall and rise in ambient oxygen saturation. (A) Heartbeat pulse amplitude and frequency before and (B) after section of the visceral nerves. *N*=3. (C) Results with a fourth animal tested only after visceral nerve section (average from two experiments). Other details as in Fig. 2.

of experiments in which octopuses were transferred between buckets of well-aerated and oxygen-depleted water. The effects of a switch either way were found 'within seconds'; Wells (1979) show effects within one or two heartbeats. Both series of experiments included transfers to water that had been oxygen-depleted by boiling, showing that the response was to changes in oxygen tension, not to accumulated metabolites.

Long-term effects of visceral and branchial nerve section on the response to increased oxygen demand caused by feeding

In a series of experiments with five small animals (mass 23–61 g), in closed respirometers, oxygen uptake was measured before any operation, after section of the vertical mantle septum, after bilateral section of the visceral nerves and, in three instances, after additional bilateral section of the

Table 1. *Experimental results for octopuses before and after operations*

Animal	Intact	Septum cut	Visceral nerves cut	Branchial nerves cut						
XB1										
$\dot{V}_{O_2}$	F*196	–	S89; 101	S80	F*195	F121	F159	F109	F131	S61
Mass	23.3	–	26.5	22	22	23.7	24.9	27.5	27.7	27
Date	2/8	–	3/8	10/8	13/8	14/8	16/8	17/8	19/8	24/8
XB2										
$\dot{V}_{O_2}$	S130	S106	S120; 111	S85	–	F108	F104	F139	F140	S58
Mass	46	46	39.9	35.5	–	36.5	39	40.9	42.7	38.3
Date	3/8	4/8	5/8; 6/8	10/8	–	16/8	17/8	18/8	19/8	24/8
XB3										
$\dot{V}_{O_2}$	S103	S113	S94	S82	F138	F103	F118	F113	F96	S61
Mass	68.5	63.3	63.3	54	54	53.7	62.2	62.2	59.2	54.9
Date	3/8	5/8	6/8	10/8	12/8	14/8	16/8	17/8	19/8	24/8
XB4										
$\dot{V}_{O_2}$	S114	S104	S94; 73	–	F151	F103	F114	F120	F97	S50
Mass	61.4	59.6	59.6; 54.8	–	54.8	57	59.7	62	63.4	57.8
Date	4/8	5/8	6/8; 10/8	–	12/8	14/8	16/8	17/8	19/8	24/8
XB7										
$\dot{V}_{O_2}$	S108	S103	S62; 59	–	F164	F111	F104	F105	F97	S48
Mass	23	23	23; 22.3	–	22.3	23.4	24.6	24.6	25.3	22.3
Date	5/8	6/8	9/8; 11/8	–	13/8	14/8	16/8	17/8	19/8	24/8

Rates of oxygen consumption, mass and experimental data for five small octopuses before and after operations in which the vertical mantle septum and then the visceral nerves were cut. Three of the animals had a third operation in which the branchial nerves were cut.

Each entry shows whether the animal had been fed (F, fed on the evening before the experiment; F*, fed approximately 1 h before the experiment) or (S) starved for at least 24 h or since the last recorded date, the animal's $\dot{V}_{O_2}$ (in ml kg^{-1} h^{-1}) and mass (in g) and the date of the oxygen uptake experiment.

branchial nerves between the cardiac ganglia and the gills. After this the animals were fed over a series of days and finally starved for 5 days. The results are summarised in Table 1.

The experiments show that cutting the visceral nerves (and also severing the branchial nerves) did not prevent subsequent large increases in $\dot{V}_{O_2}$ when the animals were fed. The $\dot{V}_{O_2}$ of the five animals averaged 76±5 ml kg^{-1} h^{-1} (S.E.M.) on the day before and 151±14 ml kg^{-1} h^{-1} on the day after feeding [140±12 ml kg^{-1} h^{-1}, if the very high rate shown by XB1, which may have included an element of specific dynamic action (see Wells *et al.* 1983*b*), is excluded]. High rates of oxygen uptake were maintained while the animals were fed daily (average 114±4 ml kg^{-1} h^{-1}). 12 h after the final feed, mean $\dot{V}_{O_2}$ was 112±10 ml kg^{-1} h^{-1}, falling to 56±3 ml kg^{-1} h^{-1} after 5 days of fasting. These results show that large changes in the rate of oxygen uptake can take place after section of the visceral and branchial nerves, implying large changes to ventilatory and cardiac outputs independent of signals from the gills/hearts complex. They imply, moreover, that cardiac output can be raised without a nervous input from the brain.

Attempts to poison the oxygen receptors with cyanide

In fish, under normoxic conditions, injection of a bolus of NaCN into the ventilation stream causes increased P_V and f_V and produces a transient bradycardia. Injection of a bolus of NaCN into the bloodstream causes an increase in P_V and f_V, without bradycardia. The internal receptors concerned appear to be in the gills because injection of cyanide into the ventral aorta produces a much more rapid response than injection into the dorsal aorta (Burleson and Smatresk, 1990).

Attempts were made to repeat these experiments with *Octopus*. Burleson and Smatresk did not state the dose rate per kilogram given to their animals. The dose was apparently a standard 500 μg into the ventilation stream or 50 μg into the blood. The mass of their catfish ranged from 250 to 2000 g.

We used octopuses weighing 750–1100g. The following experiments were carried out. (1) Injection of NaCN upstream of the gills; 16 tests with four animals at dose rates of from 625 μg kg^{-1} to 6.25 mg kg^{-1}; plus four tests with three animals using KCN at 755 μg kg^{-1} to 6.25 mg kg^{-1}. (2) Injection of NaCN into a branchial heart or the dorsal aorta; nine tests with six animals at 95 μg kg^{-1} to 5.9 mg kg^{-1} and five tests with

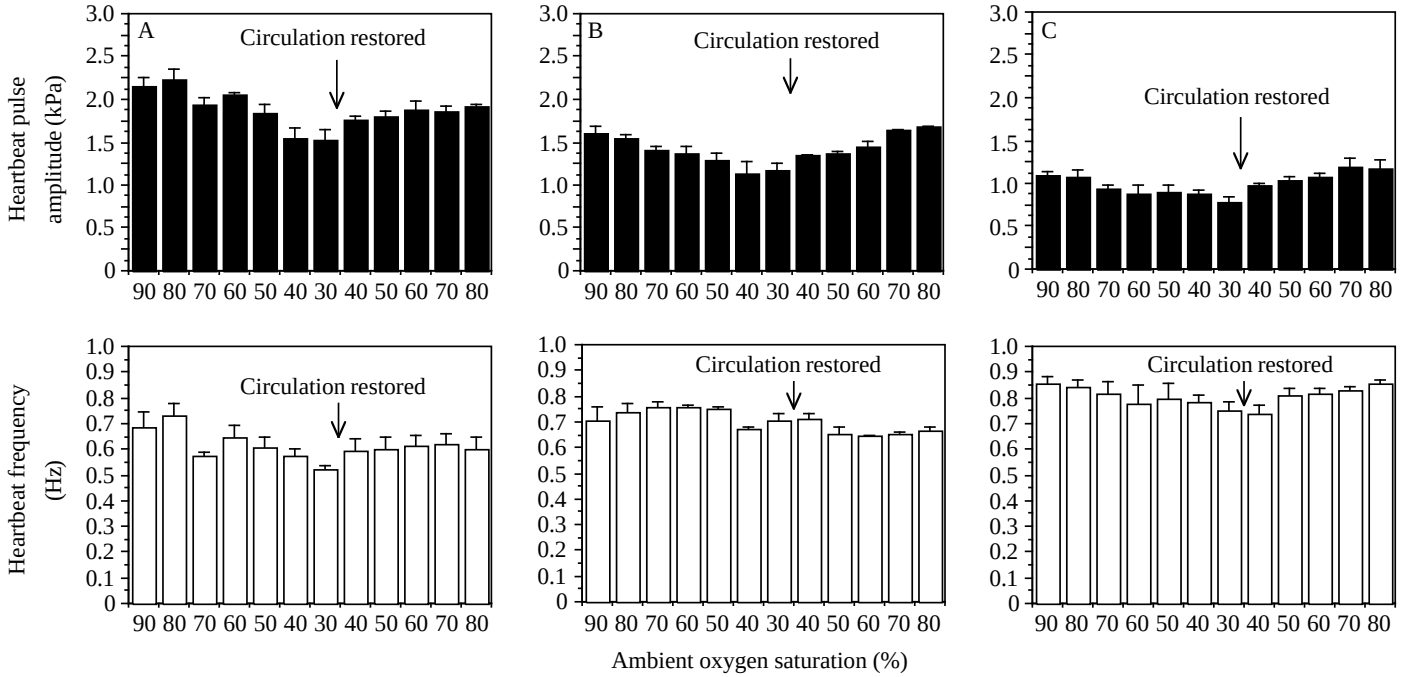

Fig. 8. The response of the systemic heart to a fall and rise in ambient oxygen saturation. (A) Heartbeat pulse amplitude and frequency before and (B) after section of the branchial nerves. *N*=2. (C) Results with a third animal tested only after section of the branchial nerves (average from two experiments). Other details as in Fig. 2.

three animals using KCN at 430 μg kg^{-1} to 3.3 mg kg^{-1}. The cyanide was typically delivered in a 0.25–1.0 ml bolus of sea water, larger doses in the higher volumes. The external dose rates given thus ranged up to 10 times and the internal dose rates up to 100 times those found effective in fish. In no case was an effect of NaCN or KCN observed.

Discussion

The control of ventilation

Ventilation pulse pressure (and, to a smaller degree, ventilation frequency) increases progressively as the ambient P_{O_2} falls from 18.6 to 6.7 kPa. P_V and f_V return to normoxic values when a circulation is restored and the P_{O_2} returns to 18.6 kPa. The receptors concerned must be within the hearts/gills complex because the responses to changes in ambient P_{O_2} cease if the visceral nerves are cut (Fig. 2). The principal receptors must be in the gills because the responses also disappear, or are very much reduced, if the branchial nerves are cut (Figs 3, 4).

A second line of evidence indicating that the receptors detecting ambient oxygen levels must be within the hearts/gills complex comes from the speed of the ventilatory response. Figs 5 and 6 show that the response to a change in ambient P_{O_2} begins within one or two ventilation cycles. The heart rate of *Octopus vulgaris* is typically faster than the ventilation rate. In the present series, the mean heart rate of control animals in normoxic water was close to 0.7 Hz (Figs 7, 8), corresponding to a ventilation rate of 0.4 Hz (Figs 2, 3, 4). But the heart rate slows (Wells and Wells, 1983, 1986) and the ventilation rate speeds up as the ambient P_{O_2} falls; in the present series, f_H fell to 0.6 Hz at 30 % saturation, while f_V rose to 0.55 Hz. At the very low (1.7–3.5 kPa) P_{O_2} used to investigate the speed of response to well-aerated water (see *The speed of response* above), f_V actually exceeds f_H (Wells and Wells, 1983).

The blood from the gills could not possibly reach internal receptors outside the hearts/gills complex within 1–2 ventilation cycles under these circumstances. The gills drain into large efferent branchial vessels, forming the systemic heart auricles, which must have a combined volume at least as great as that of the heart that they fill, so that blood from the gills will not reach the dorsal aorta to pass outside the area served by the visceral nerves until the second systolic beat following any change in ambient P_{O_2}. Even this supposes that the blood P_{O_2} or oxygen content changes instantly as the ambient stream arrives at the gills, which seems unlikely in view of the normal residence time of about three heartbeats (Eno, 1987) and the the six or so heartbeats needed for drugs injected into an afferent branchial vein to pass through the gill and efferent branchial artery and change the systemic heartbeat (Wells, 1983; Wells and Mangold, 1980).

It is concluded that the ventilatory response to changes in the ambient P_{O_2} must depend on receptors in the gills.

Control of the heartbeat

In contrast to ventilatory responses, the response of the three hearts to hypoxia (the branchial hearts respond in the same manner as the systemic heart; Wells and Wells, 1983) seems not to be dependent upon receptors in the gills. As the oxygen tension falls, the aortic pulse pressure falls. When a circulation

of aerated water is restored, Pa returns to normal. The response is not changed by cutting the branchial or visceral nerves (Figs 7, 8).

Percentage changes to the heart rate and pulse pressure are small compared with the changes to ventilation. This is possible because of the extreme Bohr/Haldane coefficients of cephalopod haemocyanins (Lykkeboe and Johansen, 1982; Lykkeboe *et al.* 1980). *Octopus vulgaris* haemocyanin has a Bohr/Haldane coefficient of −1.58; at this level, it takes up more carbon dioxide than it sheds oxygen. As ambient hypoxia develops, arterial and venous pH both increase, raising the oxygen affinity of the blood, which remains saturated as it leaves the gills even at low oxygen tensions (Houlihan *et al.* 1982). Over the range of P_{O_2} values considered in this study, the main adaptive response to hypoxia could lie with the change in haemocyanin oxygen-affinity rather than with the response of the circulation.

The limited changes to Pa and f_H that the systemic heart shows in response to changes in P_{O_2} (Figs 7, 8) occur very rapidly (Wells and Wells, 1983) so there is again no question of the involvement of signals from outside the hearts/gills complex. At present we can only assume that these changes are a direct result of low blood oxygen levels on the heart muscle itself, or possibly on the heart muscle *via* the atrial ganglia (not shown in Fig. 1; they lie between the fusiform ganglia and the systemic heart) and the nerves innervating the heart (Wells and Smith, 1987).

Are there oxygen receptors outside the hearts/gills complex?

The experiments with small octopuses (Table 1) showed increases in $\dot{V}_{O_2}$ after feeding that must imply large increases in ventilation and cardiac output. These animals had the visceral and in some cases also the branchial nerves cut. The receptors causing increased cardiac and ventilatory output after feeding cannot therefore be within the hearts/gills complex. The most probable source of heart stimulants is the neurosecretory system, lying immediately upstream of the hearts in the anterior vena cava (Alexandrowicz, 1965; see also Fig. 1). A range of cardioactive substances, including neuropeptides, has been identified here (Voigt *et al.* 1983). Crude extracts from the walls of the anterior vena cava are effective heart stimulants at low doses; less than 2% of the material extractable from a single vein, injected into a branchial heart, causes increases in branchial and systemic heart pulse pressures lasting for many minutes (Wells, 1983; Wells and Mangold, 1980).

When an octopus moves, blood pressure and pulse amplitude can more than double. This cannot be attributed to an enhanced venous return because octopuses have hydrostatic skeletons. The venous return is strangled, rather than assisted, by contraction of the muscles during movement (Wells *et al.* 1987). Since animals continue to move about, apparently quite normally, after visceral nerve section (the small animals used for the experiments summarised in Table 1, for example, continued to catch and subdue crabs), we must again assume the existence of a blood-borne stimulant to the heart, presumably again from the vena cava.

Whether the receptors triggering the presumed release of neurosecretory materials from the anterior vena cava respond to changes in the P_{O_2} or to the oxygen content of the blood is unknown. The lack of an effect from injections of massive doses of cyanide might suggest that oxygen receptors are not involved, but cyanide injection into the ventilatory stream also had no effect, and responses here are certainly due to changes in P_{O_2}.

We have no hypotheses to offer concerning the control of changes to ventilation that occur during and after feeding or during exercise (Houlihan *et al.* 1986), beyond reiterating that these changes cannot be determined by the oxygen content or P_{O_2} of the venous return to the hearts/gills complex.

Ventilation, as discussed earlier, can change simply as a result of arousal. In an animal where even the sight of food can lead to activation of the digestive gland and a rise in $\dot{V}_{O_2}$ (Best and Wells, 1983), it is likely that several control systems operate in parallel.

In the account given above we have described one of these.

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