Localization of receptors causing hypoxic bradycardia in trout (*Salmo gairdneri*)

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Receptors causing the cardiac response to environmental hypoxia have been located in rainbow trout (*Salmo gairdneri*). Intact, unrestrained trout, acclimated to 7 or 16°C, showed a marked increase in ventilation and bradycardia when exposed to hypoxia at their acclimation temperature. In experiments designed to locate the site of receptors causing hypoxic bradycardia, the buccal cavity of a fish was divided into two chambers by a tongue depressor, allowing oxygen tension of water flowing over each set of gills to be varied independently. Irrigating one set of gills with hypoxic water ($P_{I_{O_2}}$ = 3.5 kPa) while flowing hyperoxic water ($P_{I_{O_2}}$ = 55.2 kPa) over the other caused heart rate to fall from 42.2 ± 0.6 (± SEM) to 26.4 ± 0.5 (± SEM) beats/min after 1 min of hypoxic water flow. Dorsal aortic P_{O_2} was always above that recorded when both sets of gills were flushed with normoxic water ($P_{I_{O_2}}$ = 20 kPa). Bilateral ligation of the efferent pseudobranch artery and the pseudobranch nerve (cranial nerve IX) had no effect on the cardiac response to irrigation of one set of gills by hypoxic water. Physical removal of, or section of the nerve supply (cranial nerves IX and X) to, the first gill arch eliminated hypoxic bradycardia. The biological advantage of hypoxic bradycardia is discussed and it is suggested that gill arch receptors may function to monitor and maintain oxygen tension of blood leaving the gills.

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Les récepteurs qui déclenchent la réaction cardiaque à l'hypoxie du milieu ont été repérés chez la truite arc-en-ciel (*Salmo gairdneri*). Chez des truites intactes nageant librement et acclimatées à 7 ou 16°C, il se produit une bradycardie et une augmentation marquée de la ventilation dans des conditions d'hypoxie, à la température d'acclimatation. Dans le but de localiser les récepteurs responsables de la bradycardie hypoxique, la cavité buccale d'un poisson a été divisée en deux chambres au moyen d'un abaisse-langue, de façon à pouvoir varier de part et d'autre la pression d'oxygène de l'eau dans chaque série de branchies. L'irrigation d'une série de branchies par de l'eau hypoxique ($P_{I_{O_2}}$ = 3.5 kPa) et de l'autre série par de l'eau hyperoxique ($P_{I_{O_2}}$ = 55.2 kPa) entraîne un ralentissement du rythme cardiaque de 42.2 ± 0.6 (± $S_{\bar{x}}$) à 26.4 ± 0.5 (± $S_{\bar{x}}$) battements/min après 1 min. La pression P_{O_2} de l'aorte dorsale est toujours plus élevée que celle qu'on peut enregistrer lorsque les deux séries de branchies sont irriguées par de l'eau normoxique ($P_{I_{O_2}}$ = 20 kPa). La ligature bilatérale de l'artère pseudo-branchiale efférente et du nerf pseudobranchial (nerf crânien IX) reste sans effet sur la réaction cardiaque à l'irrigation d'une série de branchies par de l'eau hypoxique. L'ablation du premier arc branchial ou la section des nerfs crânien IX et X qui y amènent l'influx nerveux éliminent la bradycardie hypoxique. La discussion porte sur l'avantage biologique de la bradycardie hypoxique; il semble que les récepteurs situés dans les arcs branchiaux soient responsables du déclenchement et du maintien de la pression P_{O_2} du sang, à sa sortie des branchies.

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Introduction

Bradycardia is the most obvious circulatory adjustment shown by elasmobranch and teleost fish to extreme environmental hypoxia (Satchell 1961; Holeton and Randall 1967*a*, 1967*b*; Marvin and Heath 1968; Butler and Taylor 1971, 1975). Elasmobranchs show virtually no ventilatory adjustment to the hypoxia (Piiper *et al.* 1970; Butler and Taylor 1975) but, in teleosts, bradycardia occurs in spite of a large increase in gill ventilation (Randall and Shelton 1963; Holeton and Randall 1967*b*). Bradycardia results from increased vagal cardioinhibitory activity in both teleosts (Shelton and Randall 1962; Randall and Smith 1967) and elasmobranchs (Satchell 1961; Butler and Taylor 1971). Randall and Smith (1967) suggest that, in teleosts, the receptors are peripheral, located on the gills or within the buccal cavity, although their innervation is not known. In the present series of experiments we have localized the oxygen receptors and identified their innervation by occlusion of blood flow, extirpation of gill arches, and nerve section.

Butler and Taylor (1975) showed that, in elasmobranchs, heart rate attained during extreme hypoxia was temperature independent. The degree of cardiac adjustment was much greater in fish acclimated to higher temperatures because the start-

ing heart rate was higher. Studies on the heart rate response to hypoxia in teleosts have either been performed over a wide temperature range or at high temperatures (Randall and Smith 1967; Holeton and Randall 1967*b*; Marvin and Heath 1968) so that the degree of the cardiac response at low temperature, or the effect that low temperature has on the afferent or efferent nervous links of the reflex arc, is unknown. In order to investigate this, in a preliminary series of experiments we examined the heart rate response of trout to hypoxia at two temperatures, 7 and 16°C, the former temperature because it was the temperature at which the localization experiments were done and the latter to allow comparison with previous work.

Methods

Experiments were done on 38 hatchery-reared rainbow trout (*Salmo gairdneri*) varying in mass from 290 to 450 g. Animals were kept in outdoor aquaria at 7°C and indoor aquaria at 16°C and fed trout pellets twice weekly. All experiments were performed at the acclimation temperature of the fish. Electrodes and cannulae were implanted during tricaine methanesulfonate (MS 222) anaesthesia (1 : 10 000 solution) on an operating table of the type described by Smith and Bell (1964). To record heart beat two thin copper wire electrodes, insulated except at the implanted tip, were placed subcutaneously, one over the heart and the other in the dorsal musculature. The signal was amplified conventionally and displayed on a pen recorder writing on rectilinear coordinates. Rate and amplitude of breathing movements were monitored using an impedance pneumograph. Thin copper wire electrodes were implanted under the skin of each operculum and the signal displayed on the pen recorder. Ventilation rate was counted from the records over 1-min periods and amplitude of breathing was assessed qualitatively, amplitude of pen deflection at any given $P_{I_{O_2}}$ being compared with that obtained at normal $P_{I_{O_2}}$. No attempt was made to calibrate the amplitude trace or relate the recorded amplitude to ventilation volume. Blood samples (75 µl) were withdrawn through a cannula in the dorsal aorta; the cannula was inserted and held in place as described by Smith and Bell (1964). The haematocrit was measured before and after blood sampling and in no case did it fall more than 10% after sampling. $P_{I_{O_2}}$ and $P_{a_{O_2}}$ were analyzed with a Radiometer E 5046 oxygen electrode in conjunction with a Radiometer PHM 71 analyzer. The oxygen electrode was maintained at the experimental water temperature and calibrated with air-equilibrated water and nitrogen gas. $P_{I_{O_2}}$ was varied by passing the water through gas exchange columns through which nitrogen (hypoxic $P_{I_{O_2}}$), air (normal $P_{I_{O_2}}$), or oxygen (hyperoxic $P_{I_{O_2}}$) was bubbled.

To study the effects of temperature on the cardiac chronotropic response to hypoxia, spontaneously breathing fish, implanted with electrodes to monitor ECG, were placed in a Brett (1964) type respirometer maintained at the acclimation temperature. Water flow through the animal section of the respirometer was 10 cm/s before and during the experiment and fish held their position against this current with no apparent expenditure of energy.

Water was flushed through the respirometer at a rate of 1 ℓ/min, which was sufficient to maintain any desired level of oxygen tension in the respirometer. We had previously established that the resting heart rates at a given temperature under these conditions were lower than those obtained from fish held in black boxes or other types of restraint often used for this kind of experiment. Fish were allowed at least 24 h in the respirometer to recover from the effects of handling and anaesthesia. Resting values for heart rate and breathing were obtained at a $P_{I_{O_2}}$ of 20 kPa. The air-equilibrated water used to flush the respirometer was replaced with water low in oxygen and P_{O_2} of the water in the respirometer gradually declined. Heart rate and ventilation were recorded at 1.33 kPa $P_{I_{O_2}}$ steps from 20 to 4 kPa, when the flow of air-equilibrated water was started again. These variables were again measured every 1.33 kPa while P_{O_2} gradually returned to the initial level. The complete cycle of water deoxygenation and reoxygenation took about 2 h. Heart rate was calculated from a 30-s record of ECG while changes in breathing were expressed as the product of the recorded signal amplitude and breathing rate at a given $P_{I_{O_2}}$, normalized by the product obtained under resting conditions.

Twelve fish were used in experiments designed to locate the receptors causing hypoxic bradycardia. The buccal cavity was divided into two lateral chambers by a wooden tongue depressor, held in the median plane by dorsal and ventral sutures through the upper and lower lip. The fish was then placed in a holder similar to that described by Shelton (1959), the head being clamped by bars located on the supraorbital ridges and the body being wrapped in fine nylon net fixed to the side of the box. Two tubes were placed in the buccal cavity, one on either side of the buccal divider, and normoxic water flow (1 ℓ/min) was maintained over both sets of gills.

In the localization experiments one set of gills was always flushed with hyperoxic water while either hypoxic (3.5 ± 0.3 kPa (SEM)) or hyperoxic (55.2 ± 2.5 kPa (SEM)) water was passed over the other side. Because of the high water flow rate the latency between switching water solutions and arrival at the gills was less than 1 s. Water temperature was held at 7°C throughout the experiment.

Removal of gill arches, nerve section, or arrest of gill circulation was only performed on one side of the fish (referred to as the 'operated side'). The operculum on that side was reflected anteriorly to expose the gills and the operation was carried out through the opercular opening. Blood flow through a gill arch was interrupted by placing a ligature of No. 2 surgical silk around the arch at the ventral insertion on the body. Gill arches were removed by placing ligatures (No. 2 surgical silk) around the arch, both dorsally and ventrally, and excising the portion between the ligatures. The nerve supply to the first gill arch was exposed by making a small incision in the skin on the anterior aspect of its dorsal insertion on the body wall. The two nerves (glossopharyngeal IX, and a small vagal branch X) which innervate the arch were located on either side of the efferent blood vessel. Both branches were sectioned and the incision closed, although care was taken to ensure that the small branch of IX serving the pseudobranch was left intact. The side of the fish on which no operative procedures were done is referred to as the 'intact side.'

The innervation of (branch of IX), and blood supply from (efferent pseudobranchial artery), the pseudobranch were destroyed bilaterally in three fish. Bilateral destruction was deemed necessary because of the possibility of exchange of blood between the two pseudobranchs, via the internal carotid (Allis 1912), during irrigation of one set of gills with hypoxic water. The operculum was reflected anteriorly and the nerve and artery running in parallel courses toward the pseudobranch were located. A small incision was made on either side of the nerve and artery and a length of No. 0 surgical silk passed under them. A ligature which occluded the artery and destroyed the nerve was tied around both, 2 mm medial to the pseudobranch. The success of this and all other operative procedures was checked *post mortem*.

Results

The Cardiac Chronotropic Response to Hypoxia at 7 and 16°C

Trout acclimated to 7 and 16°C displayed a marked increase in ventilation and bradycardia when exposed to hypoxia at their acclimation temperature (Figs. 1A and 1B). Heart rates in the two groups of fish were not significantly different ($P > 0.05$) over the range of $P_{I_{O_2}}$ 6.66 to 4 kPa, although mean heart rate in the 16°C-acclimated group was about 13 beats/min above the 7°C-acclimated group at $P_{I_{O_2}}$ 20 kPa. At the lowest $P_{I_{O_2}}$ to which the fish were exposed, heart rate of the 16°C-acclimated group was only 5 beats/min above the 7°C-acclimated group. At $P_{I_{O_2}}$ 4 kPa, heart rate was significantly below ($P > 0.01$) the initial rate in both groups of fish. The heart rate response during return to normal $P_{I_{O_2}}$ was the mirror image of the initial decline in rate in response to decreasing $P_{I_{O_2}}$ (Fig. 1B).

Localization of Receptors Causing Hypoxic Bradycardia

Preliminary Experiments

The tongue depressor preparation was used for two reasons: firstly, a single fish could act as its own control, eliminating the need for large numbers of replicates and sham operations, and secondly, it was hoped that the effect on Pa_{O_2} of application of hypoxic water to one set of gills would be offset by perfusion of the other set of gills with hyperoxic water. In order to test the latter, dorsal aortic P_{O_2} and ECG were monitored from the time that hypoxic water reached the gills on the test side. The results are shown in Figs. 2A and 2B. Dorsal aortic P_{O_2}, when the fish was ventilated with normoxic water, was approximately 15 kPa. When hyperoxic water was poured over both sets of gills Pa_{O_2} rose to 28 ± 1.3 kPa (SEM). Replacing hyperoxic water with hypoxic water flow to one set of gills caused a fall in dorsal aortic P_{O_2}, but even after 5 min the P_{O_2} of blood sampled from the dorsal aorta was in the range of 18 to 20 kPa, well above the value obtained when both sets of gills were irrigated with normoxic water. Despite the high Pa_{O_2} throughout the period of hypoxic water flow to one set of gills, bradycardia was fully developed within 30 s after hypoxic water first reached that set of gills.

Heart rate fell from 42.2 ± 0.6 beats/min (SEM) to 26.4 ± 0.5 beats/min (SEM) after 1 min of hypoxic water flow to one set of gills. In 80 trials no

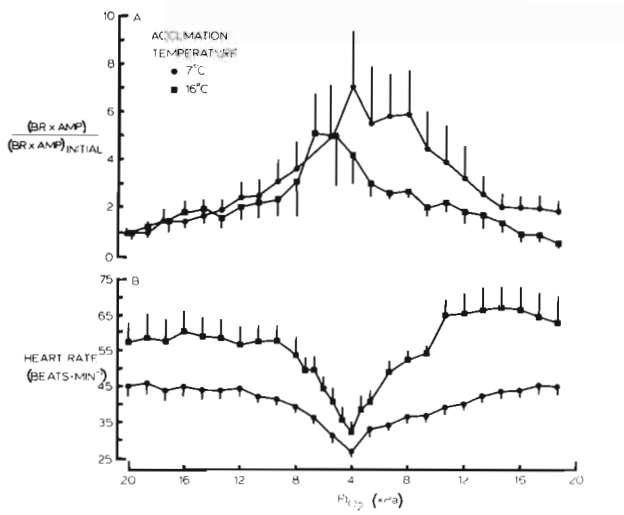

FIG. 1. The effect of environmental hypoxia on ventilation (A) and heart rate (B) in rainbow trout at two temperatures (●, 7°C; ■, 16°C). The fish were acclimated to the temperature at which the experiment was done. $P_{I_{O_2}}$ was progressively reduced and then increased, each cycle of normoxia–hypoxia–normoxia taking about 2 h. Each data point is the mean value (± SEM) obtained from 14 fish acclimated to 7°C and 8 fish acclimated to 16°C. The change in ventilation was computed as the product of the breathing rate (BR) and amplitude (AMP) at any given $P_{I_{O_2}}$ and normalized by dividing by the product obtained at normal $P_{I_{O_2}}$ ($(BR \times AMP)_{INITIAL}$).

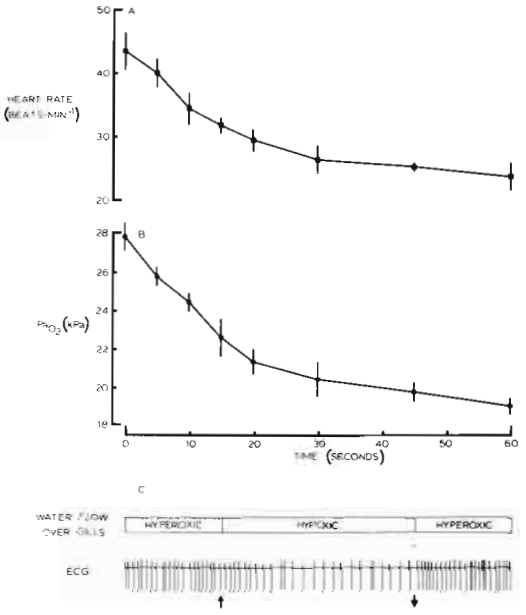

FIG. 2. The effect of irrigating one set of gills with hypoxic water ($P_{I_{O_2}}$ = 3.5 kPa), while hyperoxic water ($P_{I_{O_2}}$ = 55.2 kPa) was flowed over the other set, on heart rate (A) and dorsal aortic P_{O_2} (B). The hypoxic flow was started at time 0 and it ran until the blood sample was taken. This was repeated, on four fish, eight times. The mean of the eight values (± SEM) taken at the same time, is shown in B. The mean of the heart rate (± SEM) which pertained when the blood samples were taken is given in A. C is a tracing of an ECG record before, during, and after a period of hypoxic water flow to one set of gills (indicated by boxes over the trace and arrows under the trace).

difference was noted in the heart rate response caused by irrigating either the right or left sets of gills with hypoxic water. Results from fish in which there was a decline in the response obtained on exposing the intact set of gills to hypoxic water flow following an operative procedure on the other set of gills were discarded, as we felt that this might indicate that the fish was no longer in good condition for these experiments.

The fall in heart rate in response to hypoxic water flow over an intact set of gills (the other set being irrigated with hyperoxic water) was initially rapid; heart rate fell by some 20% in the first 10 s of hypoxic flow. Heart rate continued to fall and eventually stabilized about 37% below the initial value after 1 min of hypoxic water flow (Table 1, *a*). Restoring water flow to the test side caused an immediate increase in heart rate, the 'off response' being considerably faster than the 'on response' (Fig. 2C). Many animals exhibited a slight tachycardia 1 to 2 s after hyperoxic water reached the test set of gills; heart rate returned to the initial rate some 10–20 s later.

Bilateral Ligation of the Efferent Pseudobranch Artery and Nerve

Immediately following bilateral occlusion of blood supply from, and bilateral section of nervous innervation of, the pseudobranchs, heart rate was significantly above ($P > 0.05$) that observed in control fish (Table 1, *a* and *b*). Passing hypoxic water over one set of gills caused bradycardia with a time course similar to that elicited in intact trout. As in the control fish, heart rate fell by some 16 beats/min but, since the initial rate was higher, the rate after 1 min of hypoxic water flow was also significantly higher than in control fish ($P > 0.05$) (Table 1, *a* and *b*). In fish without functional pseudobranchs the time course of the recovery, when hyperoxic water flow replaced the hypoxic flow, was identical with that obtained in control fish.

Removal of Gill Arches and Nerve Section

Circulation of blood through all of the gill arches on one side was arrested by ventrally placed ligatures. This procedure also arrested circulation through the pseudobranch on that side. When hypoxic water was flowed over this set of gills the hypoxic bradycardia was unaffected. After tying a dorsal ligature, the gill arches were removed between the ligatures. In animals lacking a first gill arch there was no bradycardia during hypoxic water flow (Table 1, *c*), whereas leaving the first gill arch intact and removing all the others had no effect on the hypoxic bradycardia. In other experiments we attempted to localize the receptor region to a specific area of the first gill arch by tying a ligature every 2 or 3 mm from the ventral to the dorsal region of the arch. Loss of the response only occurred when the ligatures reached the dorsal portion of the arch.

TABLE 1. Change in heart rate caused by irrigating one set of gills with hypoxic water while hyperoxic water was flowed over the other set of gills. Three animals were used in each set of conditions and all values are a mean of N observations

Conditions	Heart rate ± SEM	Change in heart rate*	N
(*a*) Control			
Both sides hyperoxic	42.2±0.6		80
Intact side hypoxic	26.4±0.5	−37%	80
(*b*) Bilateral ligation of efferent pseudobranch artery and afferent nerve section			
Both sides hyperoxic	48.9±0.5		27
One side hypoxic	32.8±0.6	−33%	27
(*c*) Unilateral ablation of first gill arch			
Both sides hyperoxic	46.0±0.7		27
Operated side hypoxic	46.3±0.7	+0.6%	27
(*d*) Unilateral denervation of first gill arch			
Both sides hyperoxic	45.9±0.9		26
Operated side hypoxic	46.1±0.5	+0.4%	26

*+, up; −, down.

Section of the glossopharyngeal nerve (IX) and the vagal branch (X) innervating the first gill arch abolished the bradycardia in response to flow of hypoxic water across the set of gills on the operated side (Table 1, *d*). The response in denervated animals was almost identical with that obtained after complete removal of the first gill arch (Table 1, *c* and *d*) but, of course, in the denervated animals blood flow through the arch and pseudobranch was maintained.

Discussion

Receptors causing bradycardia during hypoxia are located on the dorsal region of the first gill arch and are innervated by cranial nerves IX or X or both. Unfortunately, the present experiments do not allow us to decide whether the receptors face the water or blood. The situation in rainbow trout is obviously very different from that found in elasmobranchs for, in the dogfish (*Scyliorhinus canicula*), receptors causing hypoxic bradycardia appear to be spread over a wide area and are innervated by cranial nerves V, VII, IX, and X (Butler *et al.* 1977).

In the present series of experiments we obtained the same degree of bradycardia, at 7°C, whether the fish was unrestrained or restrained, breathing or artificially ventilated, or whether only one or both

sets of gills were irrigated with hypoxic water. Furthermore, in unrestrained fish, dorsal aortic P_{O_2} must have fallen markedly during hypoxic exposure yet the degree of bradycardia was unaffected, indicating that the heart rate response is governed solely by receptors in the gills. However, a larger fall in heart rate occurred at the higher temperature (16°C) and others have recorded much lower heart rates during hypoxia than we provoked in the present experiments (Shelton and Randall 1962; Marvin and Heath 1968). All of these experiments were performed at temperatures above 12°C and Butler and Taylor (1975) have suggested that the sensitivity of oxygen receptors may increase with temperature so that one might expect a more pronounced response at high temperatures. Furthermore, it is interesting that the very low heart rates were obtained by others in preparations in which rhythmic breathing was absent (Shelton and Randall 1962) or appeared to have declined markedly (Marvin and Heath 1968). In trout and tench (*Tinca tinca*) coordination between heart rate and breathing rate is caused by environmental hypoxia (Shelton and Randall 1962; Randall and Smith 1967). However, the extremely low hypoxic heart rates are unlikely to be due to the lack of the stimulatory effect of breathing since, in the present experiments, there was no difference in heart rate response in spontaneously breathing or artificially ventilated animals at 7°C. In fact the latter did not display any sign of respiratory stimulation when hypoxic water was applied to one set of gills. It is most likely that the extremely low heart rates encountered under some conditions are due to a direct effect of anoxemia on the heart pacemaker.

In elasmobranchs complete denervation of receptors which cause hypoxic bradycardia has little effect on resting cardiovascular function, but this may not be the case in teleosts. Davis (1971) has shown that the first gill arch is of paramount importance for maintenance of dorsal aortic P_{O_2} in trout. Animals in which blood flow through the first gill arch and pseudobranch was arrested (bilaterally), for 12–18 h or so, had a low Pa_{O_2}, while bilateral circulatory arrest in arches 2 and 4, representing a greater restriction in gas exchange area, gave near normal Pa_{O_2}. One would presume that the receptor cells were killed by circulatory arrest for this period of time.

In our experiments selective bilateral elimination of pseudobranch function was the only procedure which affected heart rate in resting animals (of course, none of the other tests involved bilateral elimination of gill arches or nerve section). Even so the fall in heart rate during hypoxic water flow was the same as that in control fish. The fact that bilateral pseudobranch deafferentation also has no effect on ventilatory responses to hypoxia (Randall and Jones 1973) suggests that this structure is not an integral part of any hypoxic control system.

The biological advantage of hypoxic bradycardia is obscure. Heath (1964) suggests that it reduces myocardial oxygen consumption, while Holeton and Randall (1967*b*) suggest that bradycardia allows blood to remain in the gill lamellae for a greater time so that oxygen equilibration may occur in the face of a diminished gradient between water and blood. The position of the first gill arch receptors, allied to the fact that circulatory arrest in the first gill arch adversely affects Pa_{O_2} (Davis 1971), suggests that these receptors could function in maintaining blood P_{O_2}. When environmental hypoxia is sufficiently severe to cause a fall in blood oxygen tension at the receptor, an appropriate response would be to lengthen blood transit time through the gills by bradycardia. The effect of any increase in stroke volume (Holeton and Randall 1967*b*) could be minimized by recruitment of lamellae in hypoxia, thereby increasing the physiological vascular space in the gills (Jones and Randall 1978). In fact Taylor *et al.* (1977) have recently shown that Pa_{O_2}, at any given level of extreme environmental hypoxia, is lower in dogfish in which bradycardia is prevented by atropine injection or section of the vagi than in intact animals. Even so, we tend to regard the cardiac response to hypoxia obtained from these receptors as an extreme expression of the role of these receptors in fish in nature where trout rarely encounter environmental hypoxia of the degree used experimentally. Obviously this problem will have to be readdressed before a complete analysis of cardiovascular–respiratory interaction can be attempted in fishes.

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