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OXYGEN SENSITIVE AFFERENT INFORMATION ARISING FROM THE FIRST GILL ARCH OF YELLOWFIN TUNA

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Abstract. Single nerve fiber discharge was recorded from O_2 sensitive receptors in the first gill arch of the yellowfin tuna, *Thunnus albacares, in vitro.* These receptors were innervated by the vagus nerve and increased their discharge in response to decreasing perfusion rate, decreasing perfusion P_{O_2} and, in most fibers, to decreasing external P_{O_2}. Fibers responding to environmental hypoxia exhibited an exponential increase in discharge to decreasing external P_{O_2} with a sensitivity similar to that exhibited by cat carotid body chemoreceptors. Indirect evidence suggests that these receptors are located near the gill vasculature and are more sensitive to changes in arterial P_{O_2} than water P_{O_2}. Their response characteristics and hypoxic sensitivity strongly implicate them as the afferent limb in the cardiac responses and perhaps also the ventilatory responses exhibited by tuna to environmental hypoxia.

Chemoreceptors Teleost fish Vagus nerve
Heart rate *Thunnus albacares*

Environmental hypoxia has been shown to elicit both respiratory and cardiovascular responses, of varying magnitude, in all fish that have been studied to date. Whereas the ventilatory response in teleost fish is strong, consisting of increases in both respiratory frequency and tidal volume, very little response is shown by elasmobranch fish (see Shelton *et al.*, 1986, for review). Although there is some evidence to implicate the pseudobranch of teleost fish as a possible receptor site involved in the ventilatory response (Laurent, 1967, 1969; Laurent and Rouzeau, 1969, 1972; Barets *et al.*, 1970) most data implicates putative arterial chemoreceptors, possibly located within the central nervous system (Satchell, 1961; Davis, 1971; Dejours, 1973; Bamford, 1974; Holeton, 1977).

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The cardiac response to environmental hypoxia, in both elasmobranchs and teleosts, consists of an immediate, intense bradycardia accompanied by an increase in cardiac stroke volume (Randall and Shelton, 1963, Piiper *et al.*, 1970; Butler and Taylor, 1971). Often, in teleosts, these changes are evenly matched and thus produce little change in cardiac output (Holeton and Randall, 1967; Marvin and Heath, 1968). In elasmobranchs, the receptors involved in producing this response are diffusely spread throughout the orobranchial and parabranchial cavities and are innervated by cranial nerves V, VII, IX and X (Butler *et al.*, 1977). In teleosts, they are primarily confined to the first gill arch and innervated by cranial nerves IX and X (Smith and Jones, 1978; Daxboeck and Holeton, 1978; Smith and Davie, 1984).

Although the general location and innervation of the receptors involved in these cardiac responses have been described in elasmobranchs and teleosts, nothing is known of their exact location or response characteristics. The arteries supplying the first two branchial arches in these fishes are homologous with the carotid and aortic arches of higher vertebrates and it has been suggested that the phylogenetic fate of the O_2 sensitive receptors located in the branchial arches and gills, and innervated by the IXth and Xth cranial nerves in fishes may be in the discrete carotid and aortic bodies of terrestrial vertebrates (Butler *et al.*, 1977). If this were the case, the O_2 receptors located in fish gills might be expected to exhibit similar properties to those of the carotid and aortic bodies. To date, there have been no recordings made from O_2 sensitive receptors in the primary gill arches of any fish. In the present study, we have recorded afferent impulses from the Xth cranial nerve innervating the 1st arch of the yellowfin tuna, *in vitro*, in an attempt to locate O_2 sensitive chemoreceptors and, if present, to characterize their responses to hypoxia.

Materials and methods

Recordings were made from 15 yellowfin tuna, *Thunnus albacares*, (1–2 kg body weight) obtained from local fishermen and maintained in outdoor holding tanks at the Kewalo Research Facility. Animals were supplied with well aerated, running sea water (25 ± 1 °C) and fed a diet of smelt, krill and squid. For experiments, animals were netted from the holding tank, given an intracardiac injection of 4000 IU Heparine and killed by a sharp blow to the head followed by pithing. The afferent artery to the first gill arch on one side was immediately cannulated (P.E. 90 tubing) and perfused with aerated tuna saline (1.17% NaCl, pH 7.35, P_{CO_2} 12 mm Hg) containing 20 IU/ml Heparin and filtered with a 0.2 μm filter. All other vessels supplying the gill were severed but not ligated. The Xth cranial nerve (vagus) was isolated at the nodose ganglion and, along with the entire gill arch, was dissected free and suspended in a 25 °C bath of aerated tuna saline (fig. 1). The pressure head on the perfusion line was adjusted to 120 cm H_2O, slightly above normal arterial blood pressure, to ensure adequate perfusion. Actual perfusion flow was not measured. The vagus nerve was then dissected free of surrounding tissue, carefully lifted into a small circular chamber filled with mineral oil, placed

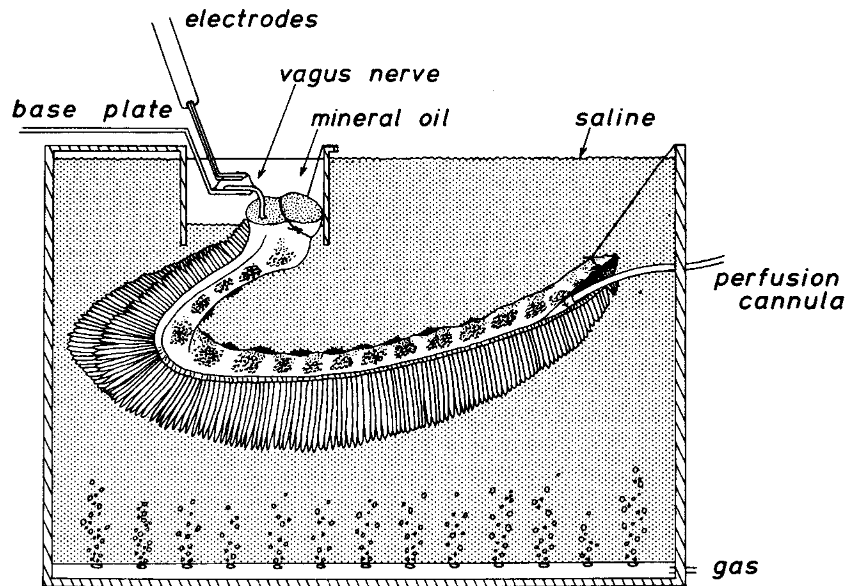

Fig. 1. Schematic diagram of experimental setup for *in vitro* recording of vagal afferent activity from the isolated, perfused gill.

on a dissecting platform (fig. 1) and desheathed. Small filaments were dissected from the distal end of a small cut made in the nerve and single unit action potentials from afferent fibers were recorded by conventional means using bipolar platinum electrodes. The activity was amplified with a Framp* PRA-2 preamplifier (F.M. Smith, Vancouver, Canada) and Grass* P15 differential A.C. amplifier whose frequency bandwidth was set from 30 Hz to 10 kHz. The amplifier output was further filtered with a 60 Hz notch filter. The filtered output from the amplifier was monitored with an oscilloscope and audio amplifier and connected to a W.P.L.* 121 window discriminator. Both the filtered signal and window discriminator output were stored on magnetic tape for later analysis using an instantaneous rate meter (EKEG* Electronics, Vancouver, Canada) and a Gould* Integrating Amplifier averaging ENG activity over 100 msec intervals.

All afferent fibers were initially tested for O_2 sensitivity by lowering the oxygen partial pressure (P_{O_2}) of the bathing solution by bubbling N_2 through the bath. The P_{O_2} of the bathing solution could be slowly lowered in this fashion and restored by again, bubbling with air. Following this procedure the response of each fiber to brief occlusion of the perfusion cannula was determined. All fibers responding to hypoxia invariably showed a dramatic increase in activity during this manoeuver. Finally, the response of most fibers to changes in P_{O_2} of the perfusion fluid produced by bubbling N_2 through the perfusion reservoir, was also measured. Because these experiments were principally designed to test for sensitivity to changes in environmental P_{O_2}, we were restricted in

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our ability to alter perfusion flow to either on or off and perfusate P_{O_2} to only one level of hypoxia. Perfusion fluid leaving the gill arch via the severed vessels entered the bathing fluid. This led to a slow turn over of the bathing solution throughout the experiment. Although the aeration procedure used to set the P_{O_2} of the bathing solution should have rapidly mixed and equilibrated the perfusion fluid as it left the gill arch, small transient changes in P_{O_2} localized around the distal stub of the gill arch may have occurred. The levels of P_{O_2} in both the bathing and perfusion solutions were measured throughout the experiments using a Radiometer* PHM 71 Acid–Base Analyzer and Oxygen electrode.

Results

Recordings were obtained in this study from 86 single units of which 22 exhibited some degree of O_2 sensitivity.

Of the 64 fibers which were not sensitive to changes in P_{O_2} of either the bathing or perfusion solutions, over 20 exhibited a variety of bursting patterns of activity. Such activity varied from bursts of 2 to 3 spikes to bursts of several hundred spikes. As a rule, the larger the burst of activity, the longer the interval between bursts (fig. 2). These fibers did not respond to mechanical stimulation of the gill filaments and gill rakers and thus their normal stimulus modality remains unknown.

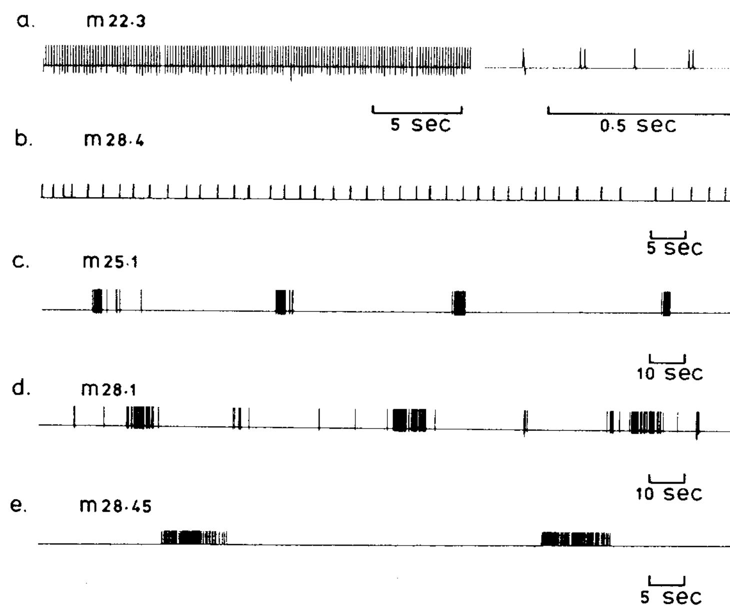

Fig. 2. Electroneurograms of afferent activity from 5 single units (a–e) exhibiting various patterns of bursting discharge under normoxic conditions. Code numbers for each fiber are included on the upper left of each trace for identification.

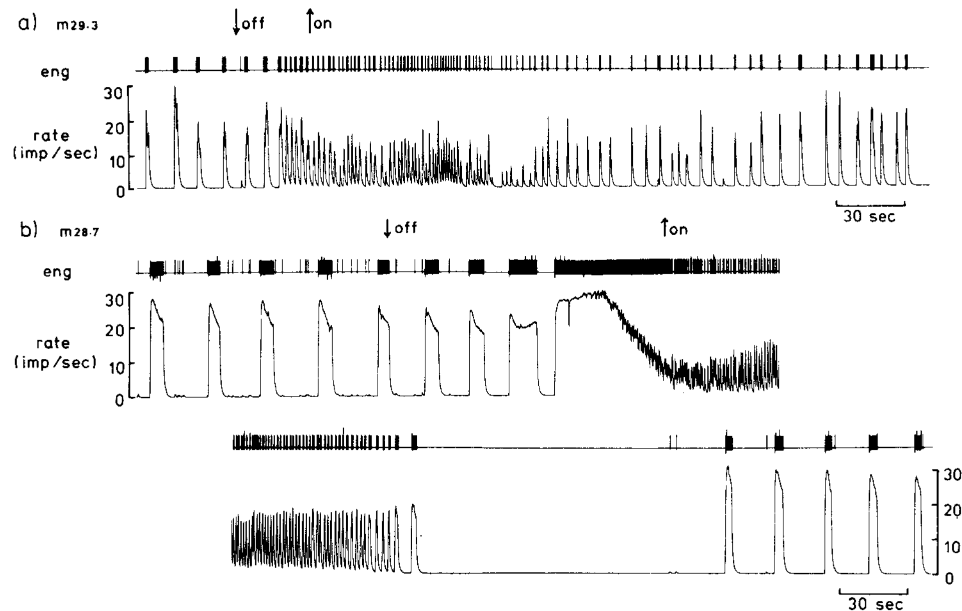

Fig. 3. Electroneurograms and instantaneous discharge rates recorded from 2 fibers exhibiting bursting activity which were sensitive to hypoxia. Perfusion of the gill was turned off and back on at the arrows. The lower two traces for the fiber shown in panel b continue on immediately from the upper two traces.

Five of the 22 fibers which were O_2 sensitive showed similar bursting patterns. The effect of transiently stopping gill perfusion is shown for two of these fibers in fig. 3. Stoppage of flow generally led to a dramatic increase in overall receptor discharge rate brought about by a great increase in burst frequency accompanied by a smaller reduction in the number of spikes per burst. No attempt was made to quantify the responses of these fibers.

The remaining 17 fibers exhibited irregular, low level discharge under normoxic conditions. All fibers exhibited a strong response to temporary occlusion of the perfusion line which could be reproduced by lowering the P_{O_2} of the perfusion fluid (fig. 4a). Six fibers, however, showed no response to a change in the P_{O_2} of the bathing solution as long as perfusion flow and P_{O_2} were maintained (fig. 4b,c). Eleven fibers did respond to a reduction in the P_{O_2} of the bathing solution. Figure 5 illustrates the typical response of one of these fibers to both a lowering of the bath P_{O_2} as well as to a temporary cessation of perfusion. The responses of all eleven, P_{O_2} sensitive fibers are plotted as a function of bath P_{O_2} in fig. 6 and the mean response of these fibers is compared to similar responses measured for cat carotid and aortic bodies (fig. 6, insert).

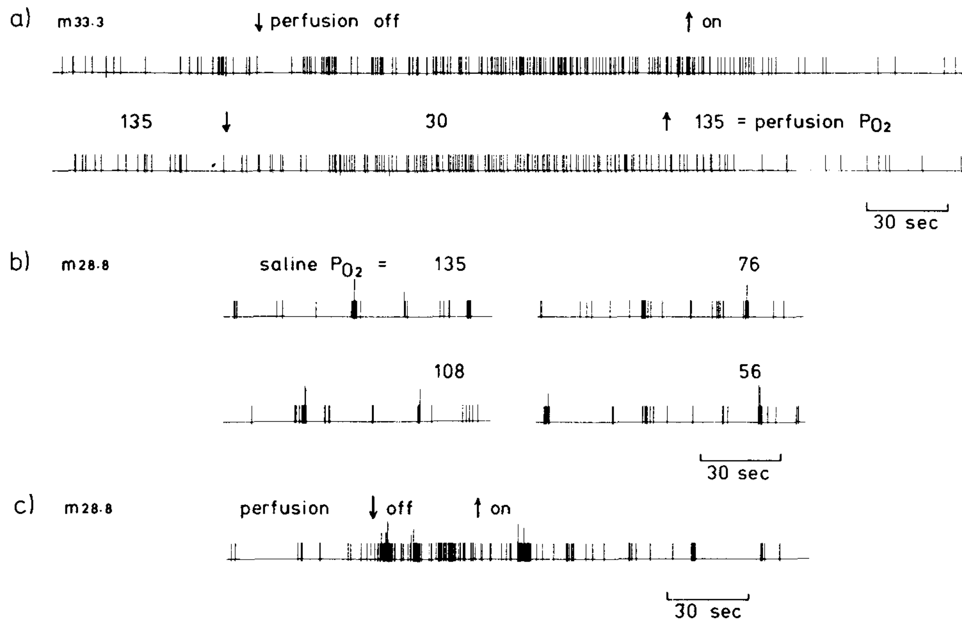

Fig. 4. (a) Electroneurograms of a single unit illustrating the response to temporarily turning perfusion flow off (upper trace) and to changing the P_{O_2} of the perfusion fluid (lower trace). The electroneurograms of a single unit which did not respond to changes in external P_{O_2} are shown in panel b while the response of the same fiber to temporary occlusion of the perfusion line is shown in panel c.

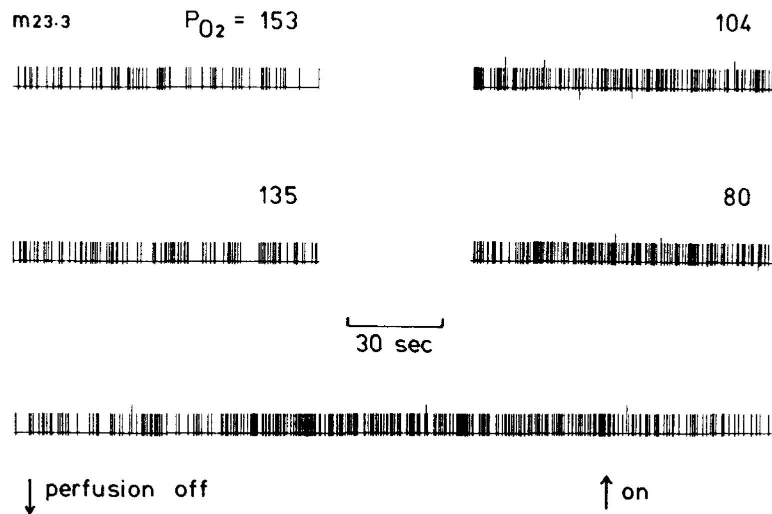

Fig. 5. Responses of a typical unit to changes in external P_{O_2} (levels indicated) are shown along with the response of the same unit to a temporary cessation of perfusion.

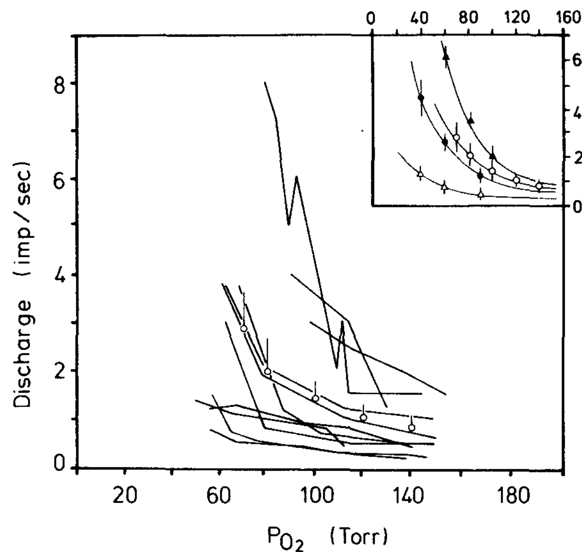

Fig. 6. The changes in discharge of eleven O_2 sensitive units are plotted against changes in external P_{O_2}. Mean values for all fibers are indicated by the open symbols. Insert: the responses of cat carotid body (▲, Mulligan *et al.*, 1981; ●, Lahiri *et al.*, 1981) and aortic body chemoreceptors (△, Lahiri *et al.*, 1981) are plotted along with those of the tuna gill O_2 receptors (○) for comparison. Vertical bars indicate ± 1SE.

Discussion

The results of this study demonstrate the presence of O_2 sensitive receptors in the first gill arch of yellowfin tuna with afferent fibers running in the vagus nerve. We found this nerve extremely sensitive to handling and thus it required more cautious manipulation than similar preparations of the vagus nerve in other vertebrate groups with which we have worked. Although it remains possible that these receptors are particular to tuna or other highly active species of fish, we feel that it was both the size and length of the afferent nerve innervating the first gill arch as well as the fact the fish were equilibrated to room temperature that contributed to the success of this preparation.

The only electrophysiological recordings reported to date of O_2 sensitive changes in afferent information arising from the branchial regions of fish were recorded from vagal branches innervating the pseudobranch in trout (Laurent, 1967, 1969; Laurent and Rouzeau, 1969, 1972). The activity recorded in these studies was characterized by slow (> 4 ms), low amplitude voltage changes which were not readily distinguishable from background noise. The authors characterized this discharge as Type B activity as opposed to a Type A activity which consisted of distinct impulses of over 50 μV with spike potentials lasting less than 2 msec. Although the spontaneous pattern of voltage changes associated with Type B activity was irregular under normoxic conditions and changed rapidly with changes in the P_{O_2} of the solution perfusing the pseudobranch, the

sensitivity of the response was extremely low, averaging only a 1% change in discharge per Torr P_{O_2} over the range from 10 to 100 Torr P_{O_2}. By comparison, the activity recorded in the present study was of a classical nature, similar to the Type A activity of these earlier workers, and showed a hyperbolic relationship between discharge frequency and P_{O_2}. Although measurements were not obtained at P_{O_2} levels below 60 to70 Torr for most fibers, extrapolation of the relationships obtained at higher P_{O_2} would give a mean sensitivity for these receptors approximately an order of magnitude greater than that recorded from the trout pseudobranch. These response characteristics recorded from the eleven, non-bursting fibers which were sensitive to environmental P_{O_2}, in the present study, are similar to those reported for cat carotid body chemoreceptors over this same range of P_{O_2} (fig. 6, insert) (Lahiri *et al.*, 1981; Mulligan and Lahiri, 1981).

The bursting discharge which was so prevalent in many of the afferent fibers recorded from in this study is intriguing. Although several recordings revealed fibers exhibiting frequent, small bursts of activity, most showed substantial bursts of over 50 spikes, the bursts occurring from 10 sec to 1 min apart. The normal stimulus modality of most of these fibers does not appear to be low O_2 or mechanical deformation of the gill filaments. Fish gills are known to show a high degree of intrinsic vasomotion (Satchell, 1962) and thus a variety of stimuli associated with smooth muscle contraction/relaxation, changes in vessel flow or local changes in blood gas or metabolite concentration remain as possible sources of rhythmic stimuli. Approximately 20% of the bursting fibers recorded from were sensitive to lowered P_{O_2} in either the bathing or perfusion fluids. Whether the bursting phenomena seen in these fibers under normoxic conditions represents a rhythmic change in local perfusion and hence cyclic changes in local P_{O_2}, also remains speculative.

Although the evidence is indirect, the data suggests that the most likely location of the O_2 sensitive receptors recorded from in this study is in close association with the gill vasculature. To begin with, although all O_2 sensitive receptors (bursting and non-bursting) were sensitive to changes in perfusion flow or perfusion fluid P_{O_2}, not all were sensitive to changes in the P_{O_2} of the bathing solution if adequate perfusion was maintained. Secondly, the speed of response of these fibers to changes in perfusion flow or P_{O_2} was extremely rapid, regardless of levels of P_{O_2} of the bathing solution. Their response (when present) to changes in the P_{O_2} of the bathing solution, on the other hand, was generally slow. The rapid and dramatic increase in receptor discharge associated with cessation of perfusion or reduction in perfusion fluid P_{O_2} further suggests that the metabolic rate of receptor cells or surrounding tissue is high and thus local P_{O_2} falls rapidly when perfusion flow decreases. As with the overall sensitivity of these receptors to P_{O_2}, these additional characteristics are also similar to those of mammalian carotid body chemoreceptors (Fidone and Gonzalez, 1986). Taken together with the phylogenetic evidence indicating that the branchial artery supplying the first gill arch in teleosts gives rise to the carotid artery of mammals, this evidence lends strong support to the hypothesis that the O_2 sensitive chemoreceptors located in the first gill arch of teleosts are homologous to the carotid body chemoreceptors of higher vertebrates (Butler *et al.*, 1977).

The rapid ventilatory responses to changes in environmental P_{O_2} exhibited by tench and trout suggests the presence of a chemosensor monitoring environmental P_{O_2} in these species (Eclancher, 1972; Bamford, 1974), while the sluggish response (1–2 min) of *Torpedo* and the sea raven suggest an internal chemosensor (Saunders and Sutterlin, 1971; Hughes, 1978). Other experiments designed to measure the response times to perfusion of presumed reflexogenic zones (Saunders and Sutterlin, 1971) as well as intravascular injection of cyanide or oxygenated and deoxygenated blood (Bamford, 1974; Eclancher and Dejours, 1975) have been equally equivocal. More or less by default, several investigators have concluded that the O_2 receptors lie within the CNS (Saunders and Sutterlin, 1971; Bamford, 1974). The electrophysiological studies of Laurent and co-workers mentioned earlier, as well as histological studies, have implicated the pseudobranch as a site of chemoreceptors involved in control of ventilation in teleosts and elasmobranchs (Laurent, 1967, 1969; Laurent and Rouzeau, 1969, 1972; Barets *et al.*, 1970) but destruction of the pseudobranch in teleosts does not eliminate the respiratory responses to hypoxia (Bamford, 1974). In fact, such responses do not disappear, although they are modified, by transection of the entire 9th and 10th cranial nerves in teleosts (Saunders and Sutterlin, 1971). Thus, until similar studies are performed on tuna, it is not possible to speculate on whether the receptors recorded from in the present study play a role in the ventilatory responses of tuna to environmental hypoxia or not.

The source of receptive sites involved in the cardiac responses to environmental hypoxia are much better delineated. In elasmobranchs they are diffusely scattered throughout the orobranchial and parabranchial cavities and innervated by the 5th, 7th, 9th and 10th cranial nerves (Butler *et al.*, 1977). In teleosts, they appear restricted to the first gill arch and are innervated by the 9th and 10th cranial nerves (Daxboeck and Holeton, 1978; Smith and Jones, 1978; Smith and Davie, 1984). Recent descriptions of a widespread system of neuroepithelial cells in the primary lamellae of all gill arches in teleosts, however, suggest that O_2 sensors may be located more widely than just the first gill arch (Dunel-Erb *et al.*, 1982). Because of limits to the number of fish available for the present study, no attempt was made to record from other branches of the vagus innervating gill arches II to IV in the tuna. The innervation and response characteristics of the receptors recorded from in the first gill arch, however, certainly implicate them as an afferent limb for the cardiac responses shown by this species to environmental hypoxia.

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