

**A POTENTIAL ROLE FOR NEUROEPITHELIAL CELLS OF
THE GILL IN O₂ SENSING**

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Introduction

Specialized O₂-chemoreceptive cells in higher vertebrates, such as glomus cells of the carotid body and neuroepithelial bodies of the lung, respond to hypoxia with a decrease in outward K⁺ current followed by Ca²⁺-dependent neurotransmission (López-Barneo *et al.*, 1988; Youngson *et al.*, 1993). These responses are thought to initiate reflex hyperventilation and other physiological adaptations to hypoxia. In lower vertebrates, *e.g.* fish, hypoxia has profound effects on ventilation, heart rate and branchial vascular resistance, but the O₂-chemoreceptor cells mediating these responses have not been identified.

Extracellular recordings from sensory fibres of a branchial branch of the vagus nerve indicated the presence of O₂ receptors within the fish gill (Milsom and Brill, 1986). Serotonergic neuroepithelial cells (NECs) identified in regions of the teleost gill filament exposed directly to the flow of water contained dense-cored vesicles at the ultrastructural level (Dunel-Erb *et al.*, 1982). Currently, the available evidence implicates gill NECs as the most likely candidates for O₂ chemoreceptors in fish, but validation of this hypothesis awaits electrophysiological characterization of the effects of hypoxia on these putative O₂ receptors.

The objectives of the present investigation were to identify and describe the distribution of NECs in the gill of the zebrafish, *Danio rerio*, and to initiate tests to determine whether or not these cells are O₂-sensitive. Our preliminary results lead us to propose that NECs of fish may be evolutionary precursors to O₂ chemoreceptors of higher vertebrates.

Materials and Methods

Confocal immunofluorescence

Gill arches were removed from adult zebrafish and prepared as whole-mounts. The tissue was permeabilized and treated with antibodies against serotonin (5-HT), synaptic vesicle protein (SV2) and a zebrafish neuron-specific antigen (zn-12) to label NECs and nerve fibres of the gill filaments and lamellae. Secondary antibodies were conjugated with fluorescent markers and the labelled structures were identified using confocal imaging.

Cell culture and electrophysiology

Gill cells of the filament and lamellae were enzymatically dissociated and maintained in L-15 medium. The presence of NECs in culture was verified by immunofluorescence, and Neutral Red (NR) was used as a vital marker for these 5-HT-containing cells (Youngson *et al.*, 1993). The whole-cell, voltage-clamp technique was used to monitor outward K⁺ current before, during and after a hypoxic stimulus. Cells were held at -60 mV and membrane potential was ramped from -100 mV to +60 mV over a period of 1 sec.

Results

Confocal immunofluorescence

Neuroepithelial cells expressed 5-HT and SV2 (Fig. 1A, B) and were situated in the efferent epithelium of filaments and lamellae. Two populations of NECs were distinguished: large, irregular-shaped NECs of filaments and smaller, spherical NECs of the lamellae. Both populations of NECs appeared to receive innervation from zn-12-immunoreactive nerve fibres (Fig. 1B) and contained SV2-positive synaptic vesicles near putative innervation sites.

Electrophysiology

Zebrafish gill cells labeled with NR expressed a voltage-dependent outward K⁺ current. The replacement of normoxic solution (150 mmHg) with hypoxic solution (20 mmHg) in the recording medium caused a reversible decrease in outward K⁺ current, noticeable at positive potentials during ramp depolarizations (Fig. 2A, B). The hypoxia-sensitive component of total outward K⁺ current in

NR-positive cells is represented in Figure 2A (inset) as a difference current, *i.e.* current in normoxia minus that in hypoxia.

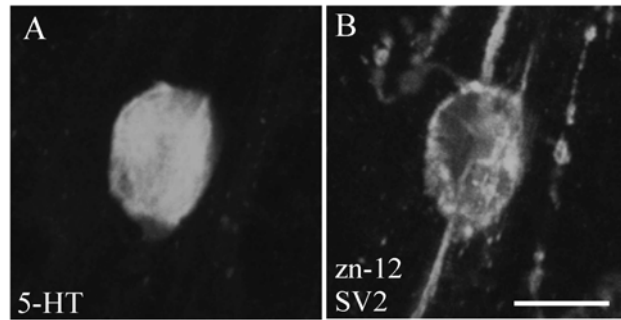


Fig. 1. Confocal immunofluorescence of neuroepithelial cells (NECs) in zebrafish gill. A, NECs are positive for 5-HT-immunoreactivity (IR). B, Same cell as in A is positive for SV2-IR and is associated with zn-12-IR nerve fibres. Scale bar 5 μ m.

Discussion and Conclusions

This study demonstrates the distribution of serotonergic NECs closely associated with nerve fibres of the gill filaments and lamellae in zebrafish. In addition, we provide the first evidence of nervous innervation of the respiratory lamellae in fish. Consistent with ultrastructural evidence from other fish species (Dunel-Erb *et al.*, 1982), the proximity of nerve fibres and the presence of synaptic vesicles localized in NECs to areas near innervation sites is consistent with a sensory role for NECs in the gill. However, an endocrine role for some NECs is not ruled out. Two distinct populations of NECs in the filament and lamellae were observed and this may reflect a difference in function between these two cell types.

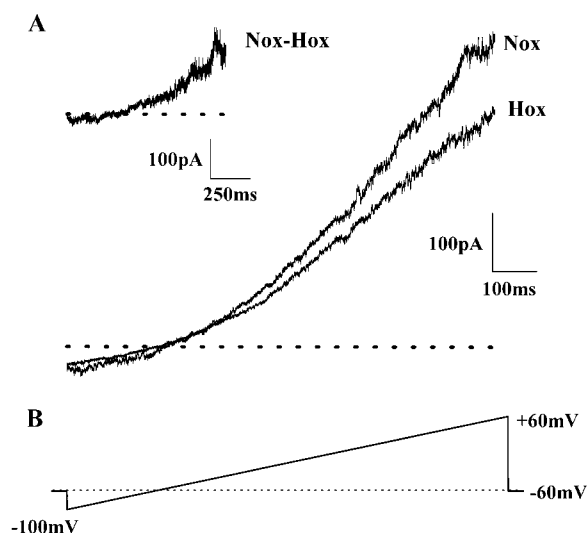


Fig. 2. Whole-cell, voltage-clamp recordings from a Neutral Red-positive cell. A, Outward K^+ current in normoxic solution (Nox) is reduced after perfusion with hypoxic solution (Hox). B, Ramp step protocol used for whole-cell, voltage-clamp recordings in A. Cells were held at -60 mV and membrane potential was ramped over 1 sec as shown.

The reversible inhibition of a voltage-dependent K^+ current by hypoxia in NR-positive, presumptive NECs of zebrafish is reminiscent of the response of specialized O_2 chemoreceptors of higher vertebrates (López-Barneo *et al.*, 1988; Youngson *et al.*, 1993). These data raise the possibility that NECs in fish may be involved in the O_2 sensing pathway and the regulation of respiration, and are phylogenetic precursors of O_2 chemoreceptors of higher vertebrates.

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