

## SHORT COMMUNICATION

# FILAMENT POSITION IN FISH GILLS IS INFLUENCED BY A SMOOTH MUSCLE INNERVATED BY ADRENERGIC NERVES

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The two rows of filaments on fish gill arches are arranged in the respiratory water current to maintain a continuous 'gill curtain'. The position of the filaments is controlled mainly by striated adductor muscles, which can draw together the filaments, and also by the elasticity of the cartilaginous filamental skeleton (gill rods) which tends to keep the filaments extended in water. In addition, the striated abductor muscles (which link the filamental rods and the gill arch bone) are arranged to increase the angle between the two rows of filaments (Riess, 1881; Bijtel, 1949; Hughes, 1984; Laurent, 1984) (see also Fig. 1).

The gill arches of teleost fish are innervated by the branchial branches of the glossopharyngeal (IX, 1st pair of gill arches) and vagus nerves (X, 2nd–4th pairs of gill arches). Although most nerve fibres in the branchial nerve trunks are believed to be sensory, motor fibres to the striated muscles and autonomic nerve fibres which control the branchial vasculature are known to be present (Nilsson, 1984).

This study was started as a fluorescence histochemical investigation of the distribution of adrenergic fibres in effector tissues of the gills of the Atlantic cod, *Gadus morhua*. It was observed that the greatest density of adrenergic nerve terminals occurred in the tissue connecting the bases of the filamental cartilage rods within the gill arch. This tissue has previously been regarded as a connective tissue ligament (transverse lamina) (Riess, 1881; Bijtel, 1949; Dornesco & Miscalenco, 1967; Hughes, 1984), but recent electron microscopical studies in the perch, *Perca fluviatilis*, have shown that this ligament is in fact a smooth muscle innervated by a-type nerve profiles (Dunel-Erb & Laurent cited in Laurent, 1984).

This paper describes the function of the adrenergically-innervated smooth abductor muscle of the cod gills. The function of another recently described adrenergically-innervated smooth muscle in the gills of some teleosts, previously also

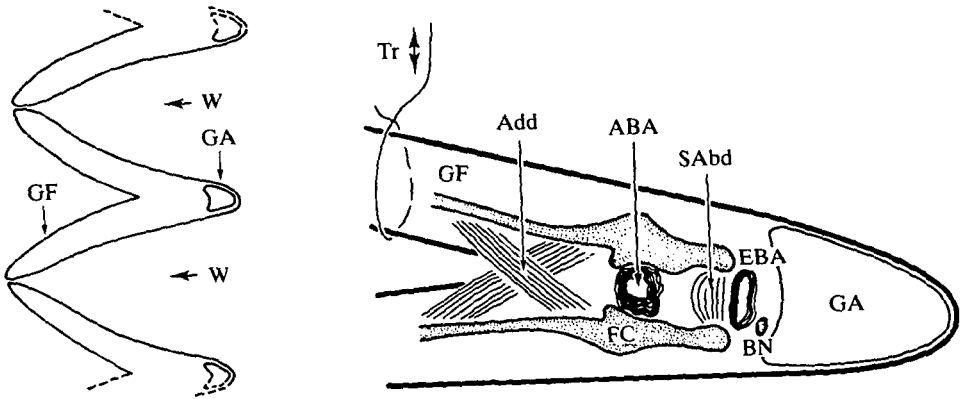


Fig. 1. The left part of the figure outlines the arrangement of the gill arches (GA) and gill filaments (GF) forming a 'gill curtain' in the respiratory water current (W). The filamental tips of two adjacent gill arches are in very close proximity in the abducted state to optimize the oxygen uptake from the water. The right part of the figure shows the arrangement of the filamental cartilage rods (FC, filamental skeleton) at the bases of two adjacent gill filaments in a transversely-sectioned gill arch. The striated adductor muscles (Add) and the smooth abductor muscle (SAbd) studied in this work are indicated. The afferent (ABA) and efferent (EBA) branchial arteries and the branchial nerve (BN) are also shown. Contraction of the smooth abductor muscle causes an increase in the angle between the two rows of filaments on the gill arch, and in the present experiments this movement was recorded using a force displacement transducer (Tr) attached to the free-moving filament.

described as a (longitudinal) connective tissue ligament (Bijtel, 1949; Dornesco & Miscalenco, 1967; Bailly, 1983 cited in Laurent, 1984) has not been investigated, nor has the system of striated adductor and abductor muscles been included in this study.

Pieces of cod gills from five animals were prepared for Falck-Hillarp fluorescence histochemistry as described by Falck & Owman (1965). The gill arch bone was removed and the tissue quick-frozen in liquid propane cooled in liquid nitrogen, freeze-dried and treated with paraformaldehyde vapour at 80°C for 1 h. Transverse sections (7 µm) were cut and viewed in a Leitz Dialux microscope equipped for fluorescence microscopy of catecholamine fluorophores. In two animals, noradrenalin (1 mg kg<sup>-1</sup> body weight) was injected intramuscularly 1 h before killing the animal. This procedure slightly enhanced the intensity of the fluorescence observed in nerve fibres.

The histochemical study revealed a dense innervation of the tissue connecting the bases of the filamental cartilage rods within the gill arch, and it is thus conceivable that the 'transverse ligament' in the cod, similar to the situation in the perch (Dunel-Erb & Laurent cited in Laurent, 1984), is an adrenergically-innervated smooth muscle (Fig. 2).

The second gill arch on the right side was dissected out and pinned to a cork dish submerged in gas-bubbled cod Ringer's solution at 10°C (see Holmgren & Nilsson, 1974). The gill arch was perfused with cod Ringer's solution at a constant pressure of 3.0 kPa through the efferent branchial artery. This ensures a nutritive circulation to the tissues of the gill arch and filaments, and allows added drugs rapidly to reach the tissues of the gill arch.

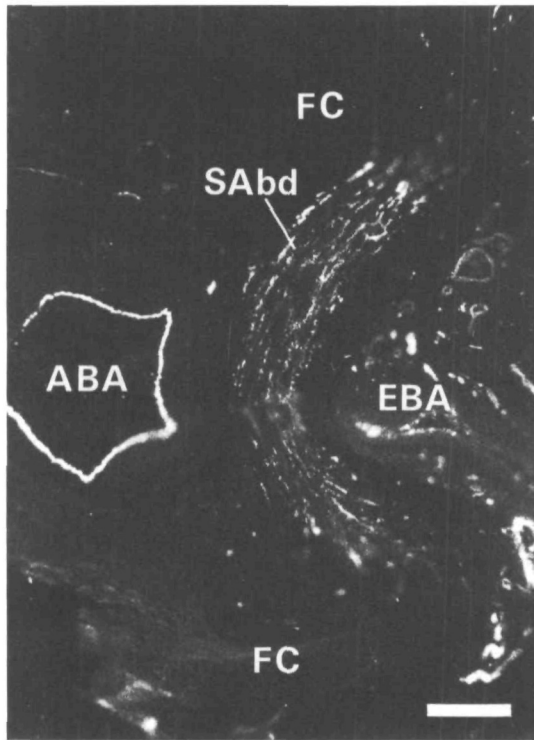


Fig. 2. Fluorescence microphotograph of a transverse section of a cod gill arch. Formaldehyde-induced fluorescence histochemistry demonstrated a very dense plexus of varicose adrenergic fibres in the smooth abductor muscle (SAbd), which links the bases of the two rows of filamental cartilage rods within the gill arch. The fluorescence of the adrenergic nerve fibres in this preparation was slightly enhanced by pre-injecting the fish with noradrenalin ( $1 \text{ mg kg}^{-1}$  body weight) 1 h prior to killing. Abbreviations as in Fig. 1. Calibration bar =  $100 \mu\text{m}$ .

Contraction of the smooth abductor muscle caused an increase in the angle between the two rows of filaments on the gill arch. In the present experiments this movement was recorded by a GRASS FT 03 force displacement transducer attached to the free-moving filament by a plastic rod with a fine platinum loop around the filament (Fig. 1). Recording of filamental movement was made using a GRASS Polygraph mod 79.

Bolus injections (see Nilsson & Grove, 1974) of adrenalin ( $10^{-9}$ – $10^{-6}$  mol in approximately 0.5 ml of perfusion fluid) into the perfusion line caused a dose-dependent abduction of the gill filaments ( $N=4$ ) (Fig. 3B). Acetylcholine ( $10^{-8}$ – $10^{-6}$  mol) added in a similar fashion ( $N=4$ ) produced adduction, probably by an action on the striated adductor muscles (Fig. 3A).

Six preparations were made in which the gill arch was dissected out with its nervous supply intact, including a part of the right sympathetic chain posterior to the vagal outflow from the skull, and perfused as described above. The sympathetic chain between the 1st + 2nd spinal nerves and the vagus was placed over

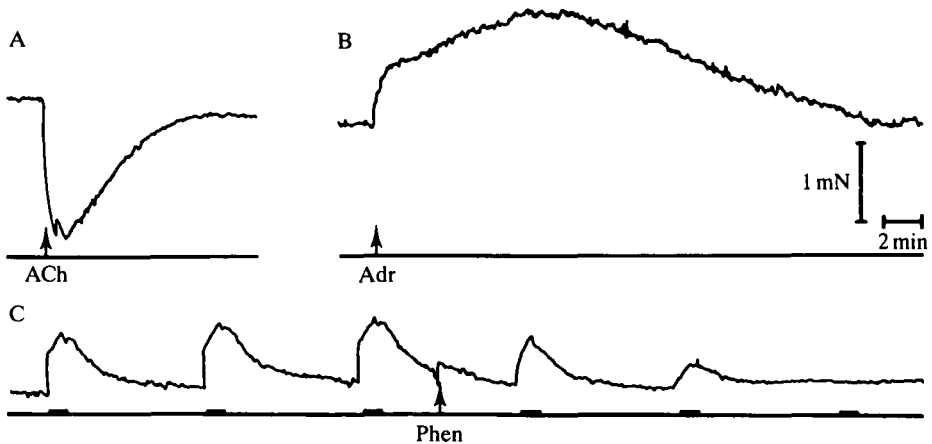


Fig. 3. Recordings of filamental movements in isolated cod gill arches caused by addition of drugs and electrical stimulation of the sympathetic nervous supply. Filamental abduction is recorded as an upward pen deflection, while adduction is recorded as a downward pen deflection. (A) Bolus injection of acetylcholine (ACh,  $10^{-6}$  mol) produced a rapid adduction of the filaments. (B) Bolus injection of adrenalin (Adr,  $3 \times 10^{-7}$  mol) produced a long-lasting abduction of the filaments. (C) Electrical stimulation of the sympathetic chain between the 1st+2nd spinal nerve and vagus nerve with trains of pulses (10 Hz, 1 ms pulse duration and 3 V) of 50 s with 8-min intervals (markings) produced filamental abduction. The response was blocked by phentolamine (Phen) ( $10^{-6}$  mol  $l^{-1}$ , dissolved in the perfusion fluid).

two platinum hook electrodes for stimulation from a GRASS SD9 square wave stimulator (for details of the nervous anatomy, see Nilsson, 1984).

Electrical stimulation of the sympathetic chain produced a marked abduction of the gill filaments ( $N=6$ ), mimicking the effect of adrenalin exposure. The abduction is attributed to a contraction of the smooth abductor muscle at the base of the cartilage rods within the gill arch. The response to nerve stimulation could be abolished by addition of the  $\alpha$ -adrenoceptor antagonist phentolamine ( $N=4$ ) ( $10^{-6}$  mol  $l^{-1}$ , dissolved in the bulk of perfusion fluid), supporting the idea of a functional adrenergic nervous control of filamental abduction in cod gills, by fibres from the sympathetic chains.

The results thus demonstrate the function of a 'new' muscular effector unit in fish gills, where active positioning of the filaments previously was thought to depend entirely on a conventional system of striated skeletal muscles. The findings have implications for all studies of branchial circulation and respiration in teleost fish where manipulation of the adrenergic system is involved, since adrenergic blockade will affect not only the branchial vasculature, and thus the gill blood flow profile, but also the positioning of the gill filaments with possible effects on the efficiency of the gas exchanger.

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