CONTRACTILE FILAMENTOUS MATERIAL IN THE PILLAR CELLS OF FISH GILLS

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SUMMARY

The secondary lamellae of the gill filaments are the sites of gas exchange in fish gills. They are mainly composed of 2 epithelial sheets joined together by pillar cells. These cells are characterized by collagen columns contained in infoldings of the cell membrane and oriented perpendicular to the epithelial sheets. The gill is the first organ to which the blood flows from the heart and within the secondary lamellae it flows through channels between the pillar cells.

The presence in the pillar cells of fine cytoplasmic filaments situated parallel to the collagen columns has now been observed in many fishes and the hypothesis has been advanced that they constitute a contractile system.

This paper describes how gill filaments were treated in a way similar to that used for other non-muscular cells in order to demonstrate in situ the presence of contractile proteins of the actomyosin type. Gill filaments were extracted in glycerol-containing solutions of low ionic strength, and then incubated with and without ATP. After incubation with ATP examination in the electron microscope showed that in the vicinity of the collagen columns, the pillar cells contain clusters of disordered thin filaments intermingled with spindle-shaped needles. This structure is characteristic of muscle actomyosin, as well as actomyosin-like proteins extracted from cells of non-muscular origin and fixed in a contracted state. It is deduced that the thin cytoplasmic filaments surrounding the collagen columns consist of an actomyosin-like contractile protein. On incubation of extracted gill filaments without ATP, the expected negative result was obtained, i.e. the filaments retain their orientation parallel to the columns. The function of the contractile filaments within the pillar cells is discussed in relation to the control of blood flow through the secondary lamellae and the reduction of pressure drop during the flow of blood across the gills of fish.

INTRODUCTION

The extensive gill surfaces of fish provide sites for the vital exchange of gases. Secondary lamellae constitute the functional units which correspond to the alveoli of the mammalian lung. Essentially these structures comprise a pair of two-layered epithelial sheets (each about 3 µm thick) which are joined by characteristic pillar cells. The blood flows through the channels between these pillar cells whose flanges line the blood channels (see Fig. 1, p. 361). The first studies of these cells using electron microscopy (Hughes & Grimstone, 1965; Newstead, 1967) revealed the presence of well-defined filaments arranged in parallel bundles. The possibility that such filaments might provide the basis for some contractile mechanism and hence the possibility of controlling blood flow and regulating the blood pressure was therefore suggested (Hughes & Grimstone, 1965). Subsequent studies on the physiology of the gills
have also emphasized the presence of mechanisms concerned with regulating the flow of blood through the secondary lamellae (Steen & Kruysse, 1964; Richards & Fromm, 1969). Thus it has become important to know whether or not the evidence for pillar cell contractility has any foundation.

It is now well established that contractility of striated muscle is based on the properties of actomyosin which may be dissociated into F-actin localized in the thin filaments of the I-band, and myosin which forms the thicker filaments of the A-band (for references see Szent-Györgyi, 1951; Huxley, 1963). Although a contractile protein complex of the same type can be extracted from smooth muscle (Needham & Cawkwell, 1956), it has only recently been shown by Nonomura (1968) and Kelly & Rice (1968) that thin and thick filaments could also be detected in this type of muscle after glycerol-extraction and incubation with ATP under conditions leading to contraction.

The glycerol-extraction method had been used originally on striated muscle fibres to study the conditions under which they contract; it allows the removal or destruction of contraction-inhibitors from the tissue, as well as low molecular weight factors such as ATP and Mg$^{2+}$, which can then be re-added in known quantities. The same technique, producing a characteristic precipitate in the presence of ATP, has enabled different authors to demonstrate the presence of actomyosin-like protein in other cells showing contractility or cytoplasmic movements. Bettex-Galland, Lüscher & Weibel (1969) were able to identify and localize in blood platelets the contractile protein which had previously been extracted from these cells (Bettex-Galland & Lüscher, 1959, 1965). Schäfer–Danneel & Weissenfels (1969), Beck, Komnick, Stockem & Wohlfarth-Bottermann (1969) as well as Keyserlingk (1969) showed the presence of 2 types of filament in fibroblasts. By several different methods of investigation, the same conclusion has been reached for other cell types showing some sort of protoplasmic contraction or movement. These include: amoebae (Pollard & Ito, 1970); leucocytes (Senda et al. 1969; Norberg, 1970); endothelial cells (Becker & Murphy, 1969; Majno et al. 1969; Puchtler, Sweat, Terry & Conner, 1969); newt and sea-urchin eggs (Miki-Noumura, 1969; Perry, John & Thomas, 1971), and slime-mould plasmodium (Nackmias, Huxley & Kessler, 1970).

The work described in this paper demonstrates by the same technique contractile protein in the pillar cells of fish gills. Some of the physiological consequences implied by the localization are discussed.

MATERIALS AND METHODS

Normal rainbow trout (Salmo gairdneri) weighing approximately 200 g were obtained from a hatchery. They were killed and their gill arches transferred to cold water; from these, 2–3 mm long gill filament tips supporting the secondary lamellae were cut with fine scissors and soaked immediately in the fixative or the glycerination solution.

Fixation without prior treatment was carried out at 0–4 °C in phosphate buffer 0.1 M, pH 7.4, first for 2 h in 2.5 % glutaraldehyde, then for 0.5 h in 1 % osmium tetroxide in the same buffer.

The solution used for glycerination was made up of equal volumes of glycerol and Na-K-phosphate buffer, 3.3 × 10^{-4} M, pH 7.0 (Kelly & Rice, 1968). The gill filaments were kept in this solution for 1 month at −18 °C. After that time the tissue was washed once at 0–4 °C, then incubated at 15 °C for 30 min in the following medium: KCl-phosphate buffer of 0.05 M ionic
strength and pH 6.8 (prepared by diluting a solution of KCl 2.7 M and Na-K-phosphate buffer 0.131 M, of a total ionic strength of 3 μ), containing 10⁻² M MgCl₂, with or without the addition of 2 × 10⁻⁴ M ATP. The incubated gill filaments were first fixed for 15 min at 0–4 °C in a solution of the same composition as the incubation medium containing 2.5 % glutaraldehyde, then for 1 h in 1 % osmium tetroxide. For the intermediate washes, incubation mixtures cooled at 0–4 °C were also used.

All samples, fresh or after extraction and incubation, were stained in block with 5 % uranyl acetate in maleate buffer, 0.05 M, pH 5.0 (Farquhar & Palade, 1965). The gill filaments were then embedded in Epon by the usual technique of Luft (1961). Oriented thin sections were made and stained with lead citrate (Reynolds, 1963).

We mainly used a Philips EM 300 electron microscope with an accelerating voltage of 80 kV, double condenser, 25-μm objective aperture and anticontamination device.

RESULTS

Structure of the pillar cell

The basic structure of the secondary lamella in trout gills is similar to that found in other fishes, previously examined. Pillar cells form clearly defined elements in sections transverse to the main axis of blood flow through the secondary lamella (Figs. 1, 2).

Each pillar cell (Fig. 2) has a central nucleus surrounded by quite dense cytoplasm containing many vesicles and organelles. One of the most obvious features is the presence of columns, which are formed as extensions of the basement membrane on either side (Fig. 3). These columns are contained within infoldings of the cell membrane (Hughes & Weibel, 1972). In the trout there are usually about 5 such columns
in each pillar cell; each column mainly contains collagenous fibrils with characteristic periodicity of 54.9 nm (S.D. = 5.74).

Parallel to the columns and close to them, clearly defined filaments are visible in the pillar cells of all fishes which have so far been investigated (Figs. 2, 3, 5). They form nearly parallel rows of filaments lying closely around the columns (Hughes & Weibel, 1972). As seen in Figs. 3 and 5A, there is a suggestion of the presence of thick filaments (c. 16 nm diameter) densely interlaced with a larger mass of finer ones (c. 8.7 nm diameter, Table 1).

| Table 1. Mean thickness (nm) of filaments from pillar cells of trout, as described in text |
|----------------------------------|----------------------------------|----------------------------------|
|                                  | Thin filaments                  | Thick filaments                  |
|                                  | Mean    | S.D.     | S.E.M. | Mean    | S.D.      | S.E.M. |
| Non-glycerinated                 | 8.746   | 1.074    | 0.339  | 15.99   | 1.64      | 0.82   |
| Glycerinated, no ATP             | 6.772   | 0.710    | 0.178  | —       | —         | —      |
| Glycerinated, + ATP              | —       | —        | —      | 13.79   | 0.874     | 0.39   |

Appearance of pillar cells glycerol-extracted and incubated with or without ATP

After glycerol-extraction and incubation with or without ATP, the pillar cells are easily recognized, for they still retain a fairly normal shape. Nuclei are clearly identifiable, as well as membranes; ribosomes are still connected to endoplasmic reticulum lamellae; the mitochondria appear swollen. The cytoplasmic ground substance is much less dense than in cells fixed without preliminary treatment.

In pillar cells incubated without ATP, filaments situated parallel to the collagen columns are still observable (Fig. 5B), although they are not oriented as regularly as before extraction and incubation.

In pillar cells incubated with ATP, parallel rows of filaments are no longer detectable, but instead clusters of intermingled thin filaments and somewhat thicker needles (c. 13.8 nm) can be observed. Although these clusters are still situated in the vicinity of the columns, they are surrounded by empty spaces, suggesting that contraction of the material had occurred (Figs. 4, 5).

In both types of incubated material, the filaments are still separated from the connective tissue fibrils by the plasma membrane enfolding the columns. This is best observed in transverse sections of the pillar cell columns (Fig. 4).

Measurements of the diameter of the thick filaments in the ATP-treated material showed that they are significantly different from the more common thin filaments of the material not treated with ATP (Table 1). These thick filaments had a similar diameter to the occasional thick filaments observable in non-glycerinated pillar cells.
DISCUSSION

Pillar cells of normal fish gills

Pillar cells are situated in an important part of the whole circulatory system, coming into very close contact with all of the blood during its single circulation. Flanges of these cells line most of the channels through which blood flows in the secondary lamellae and hence they are readily influenced by reagents transported in the blood. Changes in pillar cell length would clearly have considerable influence on the whole cardiovascular dynamics.

The pillar cell contractile material

In purified muscle extracts, contraction can be simulated by adding ATP and Mg\(^{2+}\) ions to actomyosin precipitated by lowering the ionic strength: the precipitate contracts actively upon the addition of ATP, a phenomenon termed ‘superprecipitation’. In the actomyosin superprecipitate, the same components are morphologically recognizable, although they are not oriented in parallel rows as in the sarcomeres. As stated by Pinset-Härström (1968), ‘This leads to the conclusion that for the system to show contractility the two proteins must be integrated in a specific multimolecular structure’, which for actin is the thin filamentous form, for myosin the spindle-shaped thicker needles.

Among the contractile proteins of non-muscular origin, one of the best known is the so-called ‘thrombosthenin’ isolated from human blood platelets (Bettex-Galland & Lüscher, 1959, 1965). It was shown with biochemical methods to be dissociable into an actin-like and a myosin-like component (Bettex-Galland, Portzehl & Lüscher, 1962, 1963). When examined using negative staining techniques, superprecipitated thrombosthenin shows the same features as muscle actomyosin: the actin-like protein forms very long and thin double-stranded filaments (about 6·5 nm thick), intermingled with stiff, elongated spindles of the myosin component (up to 30 nm x 0·5 μm in size). It is this spindle-shaped form of the polymerized myosin component which gives the characteristic appearance in thin sections of the thrombosthenin superprecipitate. Furthermore, it enables the contractile protein to be detected by its most important function, i.e. contractility, in glycerol-extracted blood platelets, incubated in the presence of ATP (Bettex-Galland et al. 1969).

On the basis of the evidence acquired so far, the generalization may be made that the streaming of cytoplasm, amoeboid movement and contractile properties of very many cells are due to the presence of an actomyosin-like protein. Moreover, this protein may be detected by the characteristic appearance of its superprecipitate, produced intracellularly following glycerol-extraction and incubation with ATP. This is certainly the case for the pillar cells of fish gills, where the superprecipitate is mainly formed around the columns of collagen (Figs. 4, 5c).

From the morphological point of view, pillar cells resemble smooth muscle cells: usually only one type of oriented filament can be revealed by conventional methods of tissue preparation, although there is a suggestion of the presence of a slightly thicker filament variety (Table 1). As postulated, for example, by Shoenberg (1969), it has to
be assumed that the thin filaments are the actin-like protein, and that in a relaxed pillar cell myosin occurs in a low polymeric form which cannot be detected by usual electron-microscopic techniques. In fact, it has been demonstrated more recently that smooth muscle, if fixed in the contracted state, contains thick filaments of the myosin type (Kelly & Hayes, 1969; Kelly & Rice, 1969; Keyserlingk, 1970).

Ontogeny

The presence of a contractile system having features in common with that of smooth muscle cells adds further support to the view that pillar cells are derived from smooth muscle cells (Munshi & Singh, 1968). More recently, studies of the development of trout secondary lamellae have provided evidence that pillar cells develop directly from mesenchyme cells (Tovell, Morgan & Hughes, 1970); for a fuller discussion of this question, see Hughes & Morgan (1973).

Function of pillar cells

The position of the gills in the circulation exposes them to the highest blood pressures in the body, and adaptations to reduce a fall in pressure would have the advantage of maintaining the pressure for delivery of blood to the tissues. The collagen columns probably serve to resist distension of the blood vascular channels. In order for such a system to have more than passive properties, some active contractile mechanism seems to be provided. The position of the contractile filaments close to the collagen columns is ideal for the operation of such a system.

The control of pillar cell contractility has been considered by a number of authors. There is no good evidence for any innervation, but drugs and hormones affect the resistance of the gills to perfusion through the vascular system. Östlund & Fänge (1962) showed that flow through the perfused gill apparatus increased in the presence of adrenalin and this was later confirmed by Richards & Fromm (1969). Steen & Kruysse (1964) suggested that acetylcholine favoured the flow of blood through non-respiratory pathways within the filament axis, partly resulting from contraction of the pillar cell system, adrenalin having the opposite effect. However, the evidence for blood pathways between the afferent and efferent filament vessels not involving the secondary lamellae, needs further investigation (Hughes, 1972). Rankin & Maetz (1971) have recently suggested that neurohypophysial hormones have a marked influence on pillar cell contractility in order to interpret the results of perfusion assay experiments using eel gills. A complete understanding of this mechanism will need further studies but at least the results reported here provide good evidence for the presence of a system by which pillar cells could effect such an active contraction.

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Contractile filaments in fish gill pillar cells

Fig. 2. Transverse section through a trout secondary lamella which shows a pillar cell separating 2 blood channels containing plasma (p). The tissue was fixed without prior treatment. The nucleus of an epithelial cell (e) lies opposite the pillar cell nucleus (n). These 2 cells are separated by a basement membrane which is continuous with 2 columns of collagen (c) connecting it to the basement membrane (bm) of the other side. The folding of the collagen columns suggests that the cell has been fixed in a contracted condition. The section also cuts the filaments (f) surrounding the columns. × 9250.
Fig. 3. Longitudinal section of a pillar cell column fixed without prior treatment. The basement membrane (bm), collagen fibrils (c) lined by plasma membrane (m), as well as the adjacent filaments (f) are well contrasted. Arrows point to filaments appearing thicker than average. × 37,400.
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Fig. 4. Pillar cell column in transverse section fixed after glycerol extraction and incubation with ATP. To the left of the nucleus (n), one can recognize some rough endoplasmic reticulum (rer), the cytoplasmic membrane (m) enveloping the collagen fibrils (c) cut at right angles. Around the collagen column the superprecipitated contractile protein (cp-f) is visible. × 37,400.
Fig. 5. Filament bundles or precipitate near column of collagen in pillar cell at higher magnification. *cp-f*, superprecipitated contractile protein; *f*, filaments; *m*, membrane enveloping the column. Arrows indicate filaments appearing thicker than average. × 74,500. A, fixed without treatment. B, fixed after glycerol extraction and incubation without ATP. C, fixed after glycerol extraction and incubation with ATP. The thick needles (open arrows) are clearly distinguished from the mass of thin filaments.