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The Relation Between the Form of the Action Potential and Contractions in the Subumbrellar Myoepithelium of *Chelophyes* (Coelenterata: Siphonophora)

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Summary. Action potentials of the subumbrellar myoepithelium in the diphyid siphonophore *Chelophyes* change in form with repetitive stimulation, at first showing an increasing hump or plateau on the falling phase, and finally becoming prolonged events with a large overshoot. Simultaneous records of tension developed by the subumbrellar sheet show that this change is accompanied by increase in tension. Similar prolonged action potentials to those evoked by repetitive stimulation are seen from the outset after application of Ca^{++}-blocking agents such as Co^{++}; maximal tensions are then developed from the first stimulus. The mechanism whereby change in action potential form is linked to increased tension is discussed.

Introduction

The small diphyid siphonophore *Chelophyes* (Fig. 1) swims by jet propulsion; during short bursts of jet pulses at frequencies up to 8 Hz the colony attains maximum instantaneous velocities of some 0.3 $m \cdot s^{-1}$ (Bone and Trueman, in preparation). The jet pulses are produced by contraction of the subumbrellar myoepithelium lining the jet chambers of the two nectophores. The cross-striated myoepithelial cells are coupled by numerous gap junctions, so that the subumbrellar sheet behaves as a single unit, being innervated only around the base of the nectophore (Mackie and Carré, in preparation).

An initial study of the electrical properties of this rapidly-contracting myoepithelium (Chain et al. 1981) showed that the action potentials (a.p's) propagated across it were sodium-dependent, and possessed some unusual features. When stimulated repetitively, the initial a.p's were of conventional rapidly-rising and rapidly-falling form, but subsequent a.p's showed both increased overshoot, and the formation of a plateau or hump on the falling phase leading to greatly prolonged a.p's.

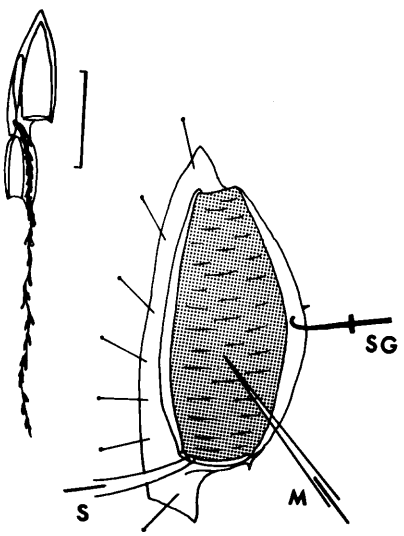

Fig. 1. Experimental arrangement, showing anterior nectophore opened (subumbrellar myoepithelium stippled). *S* stimulating suction electrode; *SG* strain gauge; *M* microelectrode. *Inset: Chelophyes* colony; *scale bar:* 1 cm

This sequence of changes in a.p. form following repetitive stimulation is seen in Fig. 2a. Essentially similar prolonged a.p's were found to be produced from the first stimulus after the preparation had been treated with Ca^{++}-blocking agents such as Co^{++}, Mn^{++}, or D-600. The effects of such changes in a.p. form upon tension development in the myoepithelial sheet were not examined; this is the subject of the present note.

Materials and Methods

Colonies of *Chelophyes appendiculata* (Eschscholtz) were collected in townets from the Rade de Villefranche during May 1981, and transferred to large aquaria in a cold room, where they survived in good condition for several days. Two kinds of experiments

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were performed. In the first, the anterior nectophore was slit open and pinned out along one side (Fig. 1), the other side being hooked to a small stainless steel entomological pin attached to a strain gauge (Dynamometer UF1). The basal nerve ring around the velar aperture was stimulated by 1–2 ms pulses from a W.P.I stimulator via a fine polyethylene suction electrode attached to the velum, and muscle action potentials were recorded via 30 Ω KCl-filled glass micropipettes. Output from the strain gauge and microelectrode amplifier led to a Tektronix 5115 storage oscilloscope. Alternatively, the whole colony was pinned to Sylgard in such a way as not to interfere with the contraction of the anterior nectophore, and a polyethylene catheter tip attached to a Millar instruments pressure transducer was used to record jet pulses, in conjunction with a Gould Brush 220 pen recorder.

Results

Histology

The organisation of the myoepithelial cells has been briefly described by Chain et al. (1981), and in more detail, by Mackie and Carré (in preparation).

The significant details for the purpose of the present note are that the cells are divided into an outer superficial mitochondrial zone, and an inner myofilament zone. The myofilament zone is penetrated by an extensive system of tubules 75–100 nm in diameter, running parallel to the myofilaments and long axis of the cell. The tubules are invaginations of the basal membrane, and are hence morphologically equivalent to a T-tubule system. In transverse section, these 'T-tubules' are more or less regularly disposed throughout the myofilament zone at intervals of some 300 nm; they contain electron-dense granular material similar in appearance to the mesogloea separating the basal membrane of the myoepithelial cells from the underlying endoderm. There are no other vesicle or tubule systems within the cells, hence the 'T-tubules' are not coupled to any other tubular system, and an equivalent of the sarcoplasmic reticulum (SR) is lacking.

This is an unusual arrangement, for although systems of T-tubules are known in other thin or small diameter striated muscle cells (for example, in larvacean caudal muscle cells), in these other muscle cells an SR system is also present, coupled to the T-system.

Physiology

Stimulation of the myoepithelium at frequencies occurring during 'spontaneous' bursts of jet pulses gives rise initially to rapidly-rising and rapidly-falling a.p's of conventional form, though without evident hyperpolarisation (Chain et al. 1981). After one or two a.p's of this kind, a hump appears on the falling phase of subsequent a.p's and eventually abolishes the original falling phase completely, giving rise to prolonged a.p's (some 50 ms) terminated by a second

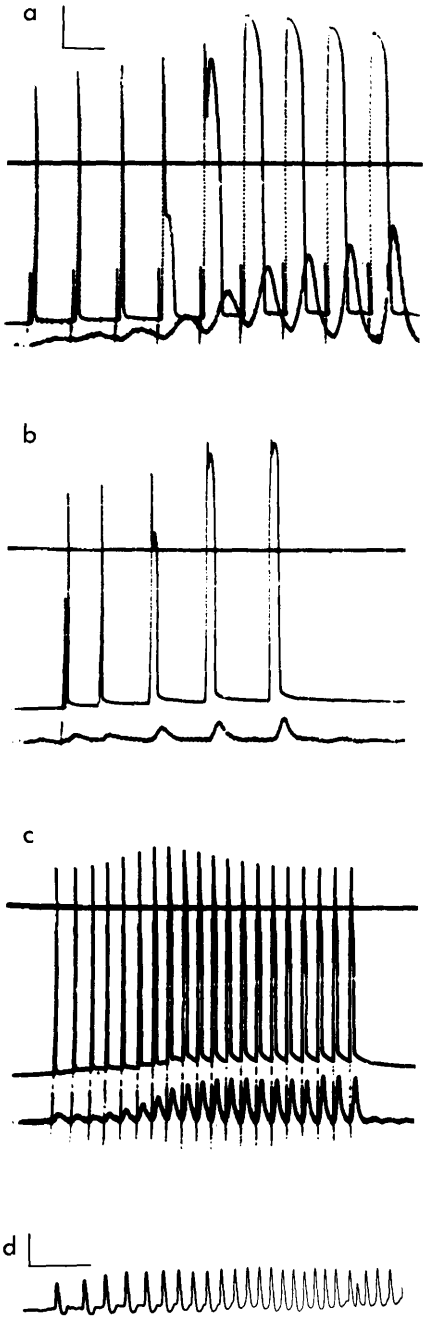

Fig. 2. a Series of stimulated a.p's from subumbrellar myoepithelium showing gradual increase in a.p. duration between 1st and 3rd a.p., and appearance of hump on falling phase of 4th a.p., increasing until a.p. is prolonged. Note increase of tension (*lower record*) as the change in a.p. form proceeds. *Scale bars:* 100 ms; 20 mV and 70 mg. **b** A single stimulus triggers a spontaneous burst of a.p's at lower frequency than **a**. Note that the change in a.p. form is not yet complete by the end of the burst. *Scale bars:* 200 ms; 20 mV and 70 mg. **c** Longer burst of stimulated a.p.'s showing that maximal tension is not achieved until several prolonged a.p.'s have occurred. The diminution in a.p. overshoot, and in resting potential during the burst, are presumably the result of increased internal Na^+. *Scale bars*: 500 ms; 20 mV and 70 mg. **d** Chamber pressures of anterior nectophore during series of jet pulses evoked by stimulation. *Scale bars:* 1 s; 500 Pa

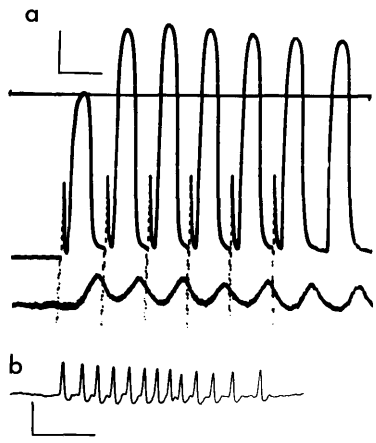

Fig. 3. a Series of a.p.'s (the last two 'spontaneous') after 15′ in sea water containing 25 mM Co^{++}. Note tension is maximal from the outset, and that the first a.p. is prolonged. **b** Chamber pressures of anterior nectophore during series of pulses evoked by stimulation following 15′ in seawater containing 25 mM Co^{++}. Note first pulse is similar to those succeeding, in contrast to Fig. 2d. *Scale bars:* 1 s; 500 Pa; 100 ms; 20 mV and 70 mg

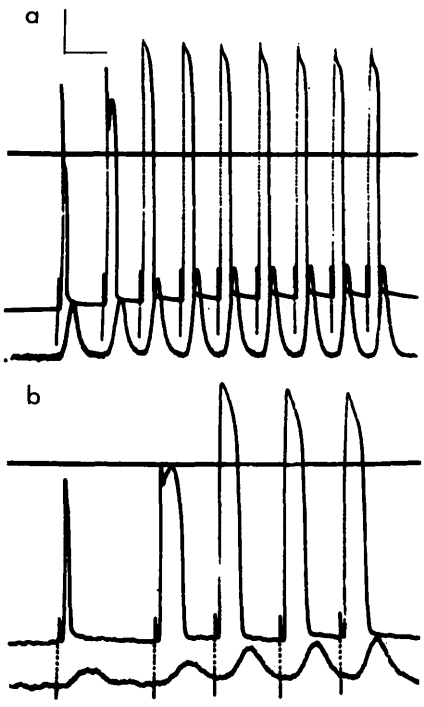

Fig. 4. a Series of stimulated a.p.'s 2 h after treatment with 25 mM Co^{++} seawater followed by repeated changes of seawater. Note first a.p. still shows a hump on the falling phase (below zero potential) and that tension now increases as the a.p.'s are prolonged. *Scale bars:* 200 ms; 20 mV and 70 mg. **b** Series of a.p.'s from the same preparation $3\frac{1}{2}$ h after treatment with 25 mM Co^{++}. Note initial a.p. now shows no hump on the falling phase, but is longer than that before Co^{++} treatment (Fig. 2a). Tension increases as the change in the a.p. proceeds. *Scale bars:* 100 ms; 20 mV and 70 mg

rapidly-falling phase. Overshoots increase from the onset of stimulation, and may be up to 70 mV (Fig. 2a). The change in a.p. form is complete after six a.p's evoked at 100 ms intervals, and succeeding a.p's are all of the prolonged form, although the overshoot may decline slightly from its highest value. At lower stimulation frequency (Fig. 2b) the change in a.p. form takes correspondingly longer (Chain et al. 1981).

Simultaneous intracellular and tension records show that maximal tensions are not developed until several prolonged a.p's have occurred (Figs. 2a and 2c). In accord with this, records of chamber pressures from intact nectophores in seawater (Fig. 2d) show that maximal chamber pressures are not developed until several jet cycles have taken place, i.e. until several a.p's have been propagated across the myoepithelium.

If now Co^{++} be added to the seawater bathing the preparation (to make a final concentration of 25 mM Co^{++} in the bath), the a.p's alter after 10–15 min so that from the outset they are prolonged (Fig. 3a). In Co^{++} solution there is evident augmentation of a.p. height on successive stimuli, followed after several a.p's by decrease in overshoot, just as in normal seawater. Despite the striking effect of Co^{++} on the a.p., contraction and tension development still take place, but tensions are now maximal from the beginning of a series of contractions (Fig. 3a). Similarly, after sojurn in Co^{++} seawater, intact nectophores produce jet pulses of the same maximum chamber pressure throughout the series; the myoepithelial contractile system is already capable of producing maximum tensions from the outset of stimulation (Fig. 3b).

The effects of Co^{++} solutions on the a.p's, and upon tension development are almost completely reversible. After 2 h of repeated changes of fresh seawater (Fig. 4a), the initial a.p. of a series still shows a slight hump, and the tension records show a small increase on subsequent a.p's.

After $3\frac{1}{2}$ h, the initial a.p. is longer than normal (cf. Fig. 2a) and the tension then developed is about half of that following several prolonged a.p's (Fig. 4b).

Discussion

Several features of the subumbrellar myoepithelium are unusual. First, it is surprising that stable intracellular records can be obtained for long periods from such thin cells. Once a cell has been penetrated, the resting potential usually remains stable for periods of hours, even during changes of the bath solution, or after repeated contractions. It seems possible that

this fortunate feature of the system may result from stabilisation of the electrode tip by the regular 'T-tubule' system. These tubules apparently contain mesogloeal material (Mackie and Carré, in preparation), and may therefore form a relatively solid meshwork inside the myofilament zone of the cells.

Secondly, the observations reported clearly indicate that tension is augmented along with the prolongation of the a.p's produced by repetitive stimuli, so that it does not attain a maximum until several jet cycles have taken place. What mechanism may be suggested to bring about this increase in tension development that is correlated with the change in form of the a.p's?

The effects of ionic changes on the a.p. (Chain et al. 1981) have shown that it is sodium-dependent. Since tension is still developed after prolonged treatment with Co^{++} solution, it is reasonable to infer that the Ca^{++} required to activate the contractile system does not enter across the superficial cell surface exposed to Co^{++} solution. Considering the manner in which the 'T-tubule' system is regularly disposed within the myofilament zone of the cell, and the absence of any other tubular or vesicle system, activation seems most likely to result from an inward Ca^{++} movement from the 'T-tubule' system. This system is unlikely to be accessible to Co^{++} in the external medium since it is derived only from the basal surface of the myoepithelial cells.

On this view, increase in tension developed as the a.p. changes form and increases in duration would result from increased Ca^{++} entry from the 'T-tubule' system during the greatly prolonged a.p's resulting from repetitive stimulation or from the effects of externally applied Ca^{++}-blocking agents. It is difficult to compare the situation in *Chelophyes* with that in other striated muscle cells, for the presence of a 'T-tubule' system in the absence of a sarcoplasmic reticulum is apparently unique. Presumably Ca^{++} entering from the 'T-tubule' system to activate the myofilaments is sequestered by the same system, so that, functionally (but not in its morphological relations), the tubular system in the myofilament zone resembles the SR of conventional muscle cells.

Finally, what role can facilitation play in the behaviour of *Chelophyes?* From the observations on colonies in large aquaria by Mackie and Carré (in preparation), and by Bone and Trueman (in preparation), it appears that short bursts of jet pulses are not only evoked by stimuli (when the rapid swimming burst can be considered an escape response), but are also generated 'spontaneously' (when they may represent a normal part of fishing behaviour). In both cases, the colony before stimulation hangs upright in the water with its fishing stem extended, and the stem is retracted (to reduce drag) as the colony shoots away with a rapid burst of jet pulses.

It is possible therefore that the slower initial acceleration consequent upon the first few pulses being less powerful than those succeeding, may permit the colony to withdraw the fishing stem undamaged before maximum instantaneous velocities are reached during the succeeding pulses.

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Chain BM, Bone Q, Anderson, PAV (1981) An electrophysiological study of a myoid epithelium in *Chelophyes* (Coelenterata: Siphonophora) J Comp Physiol 143:329–338