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CORRELATION OF ELECTRICAL AND MECHANICAL ACTIVITY OF HOLOTHURIAN MUSCLE

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SUMMARY

1. Spontaneous contractions of segments of an isolated longitudinal muscle of *Holothuria cinerascens* are not propagated across a sucrose gap. Asynchronous spiking and spontaneous depolarizations leading to contraction can be recorded independently on each side of the gap.

2. Caffeine-induced contractures are not the result of depolarization.

3. Depolarization with KCl temporarily restores contractility lost in a series of caffeine-induced contractures.

4. Acetylcholine causes a depolarization which induces contraction.

5. Individual muscle units may spike quite independently as depolarization progresses in a spontaneous contraction.

INTRODUCTION

Action potentials were recorded by Prosser, Curtis & Travis (1951) from isolated longitudinal muscles of the body wall (LMBW) of *Sclerodactyla briareus*, employing wick electrodes to detect responses to direct electrical stimulation. These responses decreased with distance or with repetition, disappearing at 2–5 mm or after 3 or 4 stimuli, an observation which was congruent with evidence that LMBW from other species is neurally activated and does not conduct waves of excitation (Henri, 1903*a*,*b*; Tao, 1927). Such action potentials could not be detected using silver wire electrodes inserted into an isolated muscle, leading to the suggestion that good contact with a number of muscle fibres may be essential for recording local action potentials in *S. briareus* LMBW (Prosser, 1954). Since non-propagating decrementing action potentials could be detected 12 mm from the site of stimulation Prosser (1954) concluded that the recorded potentials were probably not electrotonic.

In an investigation of the contractions elicited by stretch stimulation of longitudinal retractor muscles of five species of holothurians (as well as the pharyngeal retractor muscle of one species of *Cucumaria*), it was found that no muscle action potentials could be recorded using microelectrodes, polyethylene suction electrodes or glass-tipped pressure electrodes. This led to the suggestion that stretch activation

Key words: *Holothuria*, muscle, sucrose gap, asynchronous spiking.

of holothurian muscle may neurally evoke asynchronous membrane responses not detectable by means of conventional extracellular electrodes (Prosser & Mackie, 1980). However, a conventional sucrose-gap technique has been used to record the depolarizations elicited by elevated KCl concentrations in longitudinal retractor muscles of the body wall of *Isostichopus badionotus* (Hill, Sanger, Yantorno & Deutsch, 1978). These depolarizations may be expected to be large, synchronous, passive membrane responses, but it seemed worthwhile to explore the use of the sucrose gap in recording the possibly asynchronous active membrane responses which may accompany spontaneous contractions, as well as synchronous responses to pharmacological agents, in holothurian muscle.

MATERIALS AND METHODS

Specimens of *Holothuria cinerascens* Brandt 1835 were collected from beaches and reefs on Oahu and held in tanks of recirculated sea water until used (less than 6 weeks in any case). A conventional sucrose-gap technique with rubber membranes (Hill *et al.* 1978) was used to record from isolated portions of longitudinal muscles of the body wall (LMBW). Each LMBW was prepared by severing the connection to the body wall, cleaning the muscle, and cutting a segment about 8 cm long when extended. Each cleaned LMBW readily separated into two strips approximately 2·5 mm in width.

The potential difference (PD) across the sucrose gap was recorded by means of chlorided silver electrodes. Blanks without muscle tissue were run to correct for chloride sensitivity of the electrodes in experiments involving altered KCl concentrations. Alterations in PD due to chloride sensitivity were subtracted in calibration of the experimental results. Auxotonic force was recorded by means of Grass FT.03C force-displacement transducers coupled to muscle by $0{\cdot}3\,g\,cm^{-1}$ springs, with the muscles after-loaded and so disposed that they were never subjected to maintained stress, which promotes irreversible elongation (Lævin & Wyman, 1927; Galambos, 1941; Van Weel, 1955). Potential difference and force were amplified and recorded with a Grass polygraph. All experiments were performed at a room temperature of 22°C.

RESULTS

Segments of LMBW of *H. cinerascens* were mounted in the sucrose-gap apparatus under minimal stretch. Entire animals were about 15 cm long when relaxed, or 10 cm long when partially contracted. Muscles were split, cut down to about 80 mm in length, and mounted so that about 35–45 mm relaxed length was on each side of the gap. A 45-mm segment would shorten to about 20 mm in full contraction, but results are reported in terms of auxotonic force developed. Once a muscle was set up across the sucrose gap, independent spontaneous contractility developed in the two segments superfused with filtered sea water (FSW) on each side of the gap. The polarity of the recording electrodes was such that a spontaneous contraction on the

right side corresponded to a downward deflection (right electrode less positive) while a spontaneous contraction on the left side corresponded to an upward deflection (left electrode less positive). In Fig. 1, a spontaneous contraction on the left (LF) is accompanied by upward spiking which intensifies as force develops, while a spontaneous contraction on the right (RF) is accompanied by downward spiking. The spiking is interpreted as a record of asynchronous action potentials in muscle units on either side of the gap. In each case, the spiking is not transmitted across the sucrose gap, so the opposite end of the muscle serves as a reference electrode. At higher amplification, an inherent instability in the gap potential difference is evident, but does not mask a depolarization underlying the spiking, which may reflect synchronized activation of large numbers of muscle units. With the electrode orientation used, left-hand force was consistently associated with depolarization and spiking which registered upwards, while right-hand force was consistently associated with depolarization and spiking which registered downwards. For instance, in a series of 20 consecutive spontaneous contractile events (LF, RF or LF/RF) this polarity was always observed. Exceptions were never observed.

Each LMBW contracted vigorously during dissection and then gradually relaxed once set up in the sucrose-gap apparatus. At this stage, such a muscle responded to $10\,\text{mmol}\,l^{-1}$ caffeine only with very small, very slow rhythmic contractions (Fig. 2A). However, the other half of the same muscle, subjected to the same conditions and the same prior treatment, responded to $100\,\text{mmol}\,l^{-1}$ KCl with a depolarization, followed by a strong contraction (Fig. 2B). Once a muscle segment

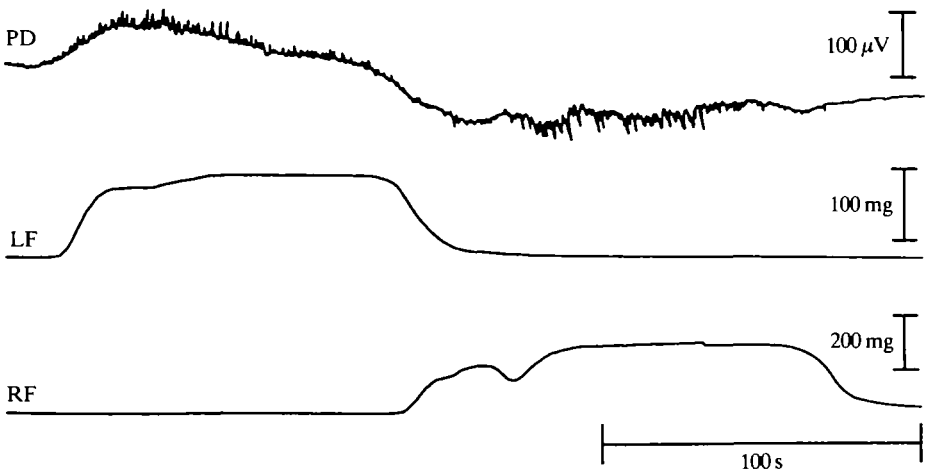

Fig. 1. Longitudinal muscle of the body wall (LMBW) of *Holothuria cinerascens* mounted across a single sucrose gap. LF, force record from the left end, superfused with filtered sea water (FSW); RF, force record from the right end, about 10 times the mass of the left end, superfused with FSW; PD, potential difference across the sucrose gap. Input G_2 on the left; inputs G_1 and ground on the right of the sucrose gap. At high amplification the baseline wanders, but spontaneous LF and RF contractions are obviously accompanied by potential variation and spikes of opposite polarity. Force is calibrated in the gravitational system, which has been conventional in muscle physiology.

had been depolarized in KCl, the first subsequent exposure to caffeine elicited contraction unaccompanied by depolarization. Subsequent exposures to caffeine elicited progressively less forceful contractions. After contractility in responses to caffeine had been essentially lost, the muscle responded to 50 mmol l^{-1} KCl with an extended depolarization and contraction (Fig. 3A). This was followed by the restoration of contractile responses to caffeine (Fig. 3B,C), although caffeine never induced depolarization.

Superfusion of a muscle portion with 10^{-5} mol l^{-1} acetylcholine (ACh) induced a relatively slow, slight depolarization, accompanied by a relatively large, brisk contraction (Fig. 4). However, the depolarization induced by ACh, although relatively slow to develop, was much larger than the depolarization accompanying a spontaneous contraction of about half the force (Fig. 4). Thus an ACh-induced depolarization is presumably more synchronized than the depolarization accompanying a spontaneous contraction. Depolarization induced by ACh was directly related to concentration (Fig. 5). The threshold for detecting an ACh-induced depolarization was around 10^{-7} mol l^{-1}, which clearly induced a contractile response. The accompanying depolarizations were difficult to distinguish from background drift. Depolarization accompanying contractile responses to 10^{-6} mol l^{-1} ACh was very small, but could be detected by inflection of the baseline in the appropriate direction. Depolarizations induced by 10^{-5} mol l^{-1} or 10^{-4} mol l^{-1} ACh were much larger, and it was evident that the onset of synchronized depolarization preceded the onset of contraction.

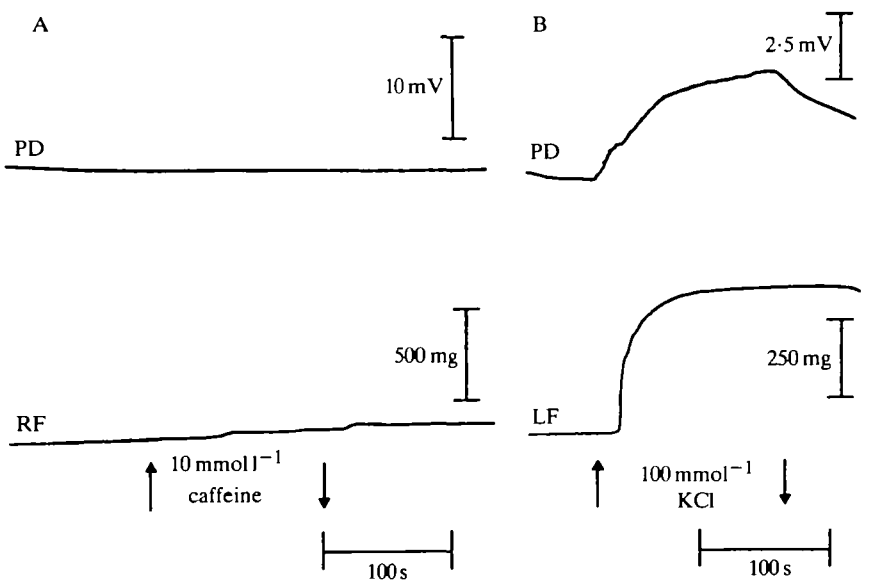

Fig. 2. *Holothuria cinerascens* longitudinal muscle of the body wall. (A) No depolarization and very little force is induced in the first exposure to caffeine of tissue on the left side of the gap. (B) Depolarization and force production are induced in the first exposure to KCl of tissue on the right side of the gap.

DISCUSSION

Electrical responses have been recorded from the LMBW of several species of holothurians. In *Sclerodactyla briareus* LMBW responses to electrical stimulation, recorded with wick electrodes, decreased in amplitude with distance up to 12 mm from the site of stimulation (Prosser, 1954), as might be expected if the isolated muscles are non-propagating (Prosser *et al.* 1951; Prosser, 1954). When communication of the LMBW of *Stichopus regalis* with the radial nerve was suppressed by cutting away the attachments to the body wall, contractions elicited by mechanical, thermal, chemical or electrical stimuli applied to a portion of the muscle were limited to a few millimetres on each side of the point stimulated (*not* propagated the length of the muscle, up to 20 cm long); similarly, stimulation of the external body surface reflexly excited a contraction only in a limited region of the underlying LMBW (Henri, 1903*b*). Isolation deprives the muscle of underlying nervous longitudinal coordination (Henri, 1903*a*; Tao, 1927). The independent, spontaneous contractions observed on the two sides of the sucrose gap in the LMBW of *H. cinerascens* in the present study may be limited to bundles of muscle fibres in which the cells communicate by the close appositions observed among fibres in a bundle (Hill *et al.* 1978; Hill, Sanger & Chen, 1982). Chen (1983) suggests that the functional units are

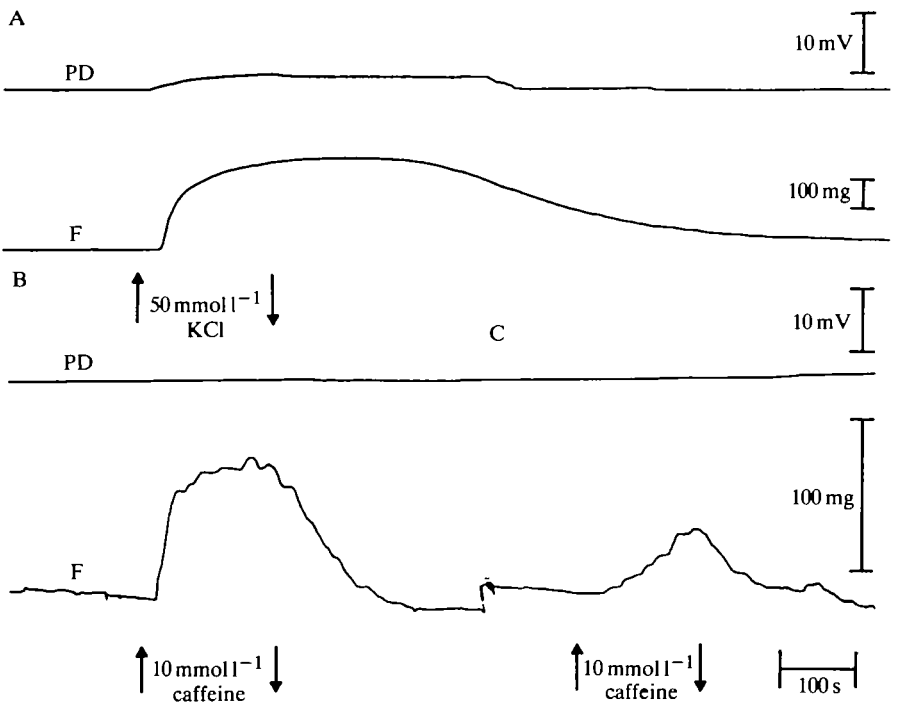

Fig. 3. *Holothuria cinerascens* longitudinal muscle of the body wall. 10 min after a small contraction (in caffeine), depolarization with 50 mmol l^{-1} KCl induced a large contraction (A). 20 min later, exposure to 10 mmol l^{-1} caffeine (B) induced a large contraction, but a second contraction (C) was slow and small. Depolarization preceded and accompanied contraction in KCl but not the contractions in caffeine.

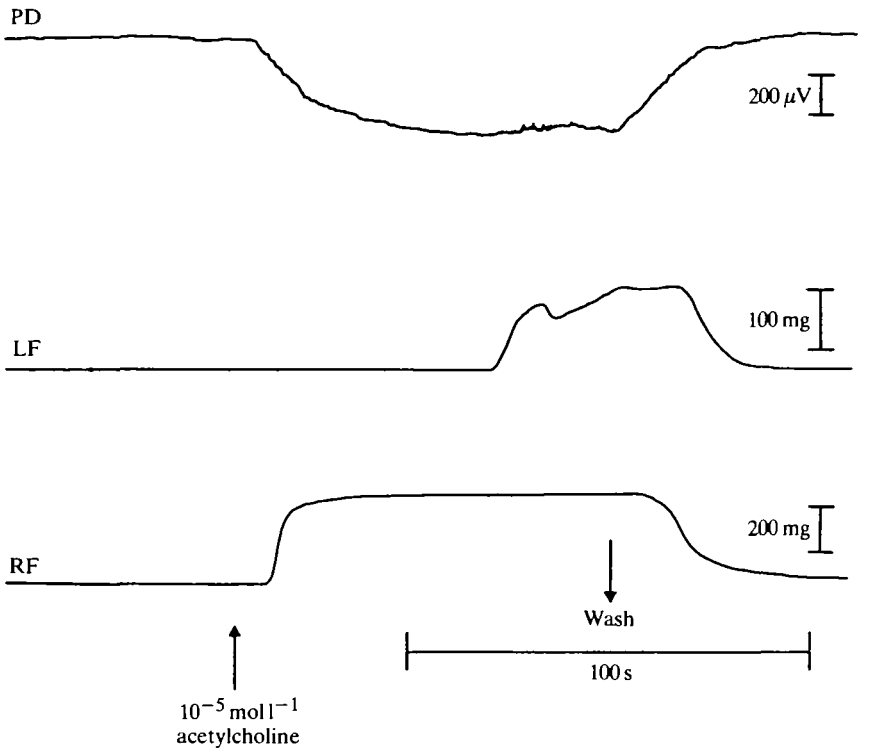

Fig. 4. *Holothuria cinerascens* longitudinal muscle of the body wall. Application of 10^{-5} mol l^{-1} acetylcholine on the right side of the sucrose gap induces depolarization (downwards) and a strong contraction on the right side, in the midst of which a spontaneous contraction on the left side occurs, accompanied by spiking and a lesser depolarization (upwards).

bundles of 1–12 fibres. Each bundle is surrounded by an external lamina, and internally linked by closely apposed thin 'frills' which project to the centre of the bundle (Hill *et al.* 1978, 1982; Chen, 1983). The cells in a bundle of holothurian LMBW have been reported to range from 1·2 to 10 μm in diameter (reviewed by Chen, 1983) and are up to 300 μm in length (Chen, 1983). Each segment in the sucrose gap thus contains a large number of small functional units. The very attenuated electrical responses observed (Fig. 1) also suggest that the active bundles are very small (Hill *et al.* 1978, 1982) and that the large amount of 'matrix' (Chen, 1983) and extracellular space (Robertson, 1980) provide a large shunting longitudinal conductance across the sucrose gap. The records of spontaneous contractions (Figs 1, 4) suggest that fibres in a bundle fire asynchronously (Prosser & Mackie, 1980), re-exciting each other to maintain a depolarization which is coupled to contraction. Responses to depolarization with KCl are probably synchronous, not depending on spontaneous activation of bundles of muscle fibres made independent by separation from the radial nerve, and are thus much larger than spontaneous depolarizing responses (Fig. 2B). Attenuation may be expected with sucrose-gap recording, but the responses to KCl obtained with *H. cinerascens* LMBW are

particularly attenuated compared to responses to KCl obtained with the LMBW of another aspidochirote holothurian, *Isostichopus badionotus* (Hill *et al.* 1978). Nevertheless, the sucrose-gap method was promising as a useful way to coordinate electrical and mechanical responses of holothurian LMBW and was used to follow up some previous observations on responses to caffeine and acetylcholine.

Isotonic mechanical recording from the LMBW of *Isostichopus badionotus* has shown a sequential loss of contractility in successive contractions evoked by 10 mmol l^{-1} caffeine (Hill, 1980). For the LMBW of *Holothuria cinerascens*, a similar sequence of exposures to caffeine results in successively less force in each contracture, and the use of the sucrose gap reveals that there is no depolarization associated with the caffeine-induced contractures (Figs 2, 3). This supports the hypothesis that caffeine releases calcium from intracellular sites in LMBW (Hill, 1980). Once contractility has declined in a series of repeated caffeine-induced contractures of the LMBW of *I. badionotus*, a brief contraction in 50 mmol l^{-1} KCl is followed by restoration of contractile responses to caffeine (Hill, 1983*a*). In *H. cinerascens*, use of the sucrose gap confirms that the contraction in KCl is accompanied by depolarization and is followed by renewed non-depolarizing contractions in caffeine (Fig. 4). This strengthens the hypothesis that KCl treatment recharges intracellular Ca^{2+} sites, by allowing Ca^{2+} entry during depolarization (Hill, 1983*a*). The contractions induced in LMBW of *I. badionotus* by 10^{-3} mol l^{-1}

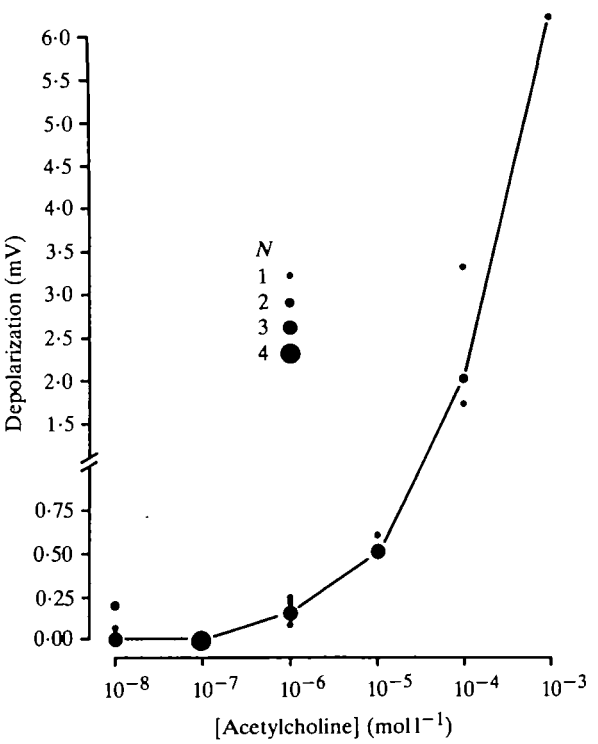

Fig. 5. Relationship of depolarization measured across the sucrose gap to molar concentration of acetylcholine, applied on either side.

ACh are blocked in Ca^{2+}-free solution, while precipitates containing Ca^{2+} have been found along the inner surface of the plasma membrane at rest, but in the myoplasm during a mechanical response to 10^{-3} mol l^{-1} ACh (Suzuki, 1982). Furthermore, contractions induced in LMBW of *I. badionotus* by 10^{-8} mol l^{-1} ACh are blocked by calcium antagonists (Hill, 1983*b*). In the present study, ACh was found to produce a depolarization (Figs 4, 5), so calcium antagonists may well block excitation–contraction coupling, as hypothesized (Hill, 1983*b*).

LMBWs of holothuria have an investment of connective tissue under the coelomic epithelium, and the bundles of muscle fibres are embedded in a thick connective tissue matrix, forming a system of septa radiating from the mid-line of the muscle mass (Chen, 1983). The septa, which delimit blocks of 3–5 muscle bundles, and the matrix of connective tissue both contain bundles of collagen fibrils and thinner bundles of elastic fibrils (Chen, 1983). Thus the slowly developing ACh-induced depolarizations probably reflect slow penetration of ACh to the centre of the muscle bundles. The relatively brisk ACh-induced contractions may begin with rapid contraction of the superficial muscle bundles.

It is of interest that the depolarizations accompanying KCl-induced, ACh-induced and spontaneous contractions fit on a spectrum with regard to amplitude and synchronization. (It is evident from the relative time course of potential and force development that depolarization gives rise to force production in all three cases.) Spontaneous contractions arise from a depolarization in the nominal 100 μV range, with superimposed asynchronous spiking. This appears to be coordinated by re-excitation of relatively independent muscle units. The force may be as large as in a KCl-induced contraction, but the onset is less smooth. The onset of KCl-induced contraction is much smoother, suggesting an imposed coordination of muscle units by depolarization in the nominal 2·5 mV range. ACh fits in the middle of the spectrum. Even a relatively high concentration, such as 10^{-5} mol l^{-1}, induces a depolarization in the nominal 300 μV range, with an irregular onset, as if individual muscle units are reacting in a relatively unsynchronized way. Consideration of this spectrum of results suggests that the least synchronized membrane effects are the most difficult to detect. If this is so, then spontaneous contractions are the least synchronized, ACh-induced contractions are intermediate, and KCl-induced contracture is the most synchronized. However, unsynchronized contractions can exert large forces.

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