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A SODIUM-DEPENDENT 'TWITCH' MUSCLE IN A COELENTERATE: THE ECTODERMAL MYOEPITHELIUM OF THE GASTROZOOIDS IN *AGALMA SP.* (SIPHONOPHORA)

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SUMMARY

Intracellular recordings were obtained from the ectodermal cells of the gastrozooids of the siphonophore, *Agalma* sp. Stimulated gastrozooids undergo rapid longitudinal contractions that are immediately preceded by a series of action potentials which propagate throughout the epithelium. These rapid potentials arise from a much slower graded depolarization. Both potentials are abolished in low sodium solutions. Fast action potentials persist in the presence of manganese ions but both the slow potentials and contraction are abolished.

INTRODUCTION

One of the most striking features of coelenterate, and particularly siphonophore, electrophysiology is that excitable epithelia, rather than neuronal pathways, are frequently used to conduct the electrical impulses which underlie the overt behaviour of these animals (Josephson, 1974; Mackie, 1965; 1978). Consequently, such coelenterate tissues are associated with a large number of different effector functions including muscular contraction (B. M. Chain, Q. Bone & P. A. V. Anderson, in preparation; Mackie, 1978; G. O. Mackie & D. Carré, in preparation), luminescence (Bassot *et al.* 1978), pure conduction (Mackie, 1965, 1978) and secretion (Mackie, 1976). Knowledge of coelenterate cellular electrophysiology is still extremely scanty, especially from the point of view of ionic studies (but see Mackie, 1976; Chain, 1979). The present investigation deals with electrogenesis in the ectodermal myoepithelium of the gastrozooids of a physonect siphonophore colony. The gastrozooids are of particular interest from a comparative point of view because of their close homology to hydroid polyps. This homology is evident from the obvious superficial resemblance of a gastrozooid to a typical polyp without oral tentacles (see Fig. 1). In addition, the structure of the body wall is that of a typical hydroid polyp (cf. Hyman, 1940). It consists of an outer ectodermal layer (forming a sheet of longitudinal muscle, and an inner, thicker, endodermal layer containing circular muscle fibres which is also highly developed for digestion. A nerve net is present in the ectodermal layer, with a particularly high concentration of nerve cells near the base of the zooid

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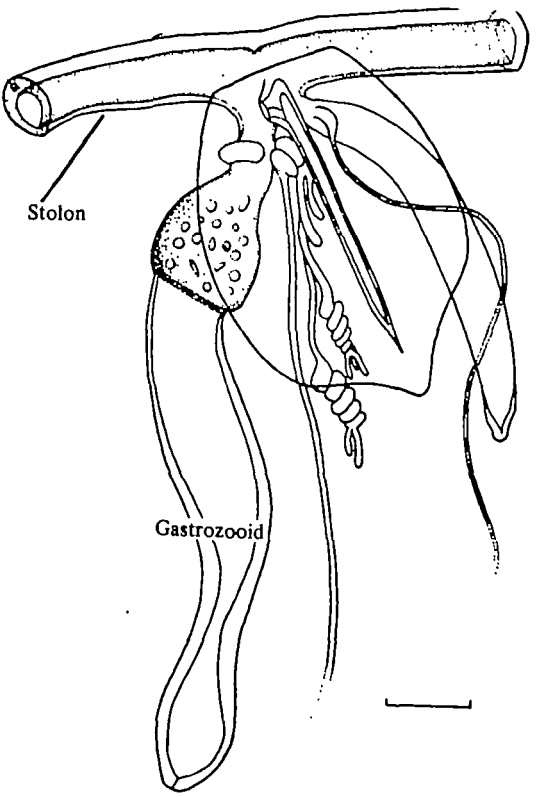

Fig. 1. A drawing of an individual gastrozooid, together with other appendages, attached to a portion of the stolon (after Mackie, 1978).

(D. Carré, personal communication). The gastrozooids, which function in the capture and digestion of food for the whole colony, are very motile. Under the experimental conditions described below they perform constant writhing movements produced by localized contractions of small areas of the body wall. In addition, electrical or mechanical stimulation can cause a rapid shortening of the whole gastrozooid which is produced by strong, symmetrical contractions of the longitudinal (ectodermal) muscles of the body wall. These contractions and their accompanying electrical correlates appear to be strikingly similar to, if not homologous with, the contraction pulse systems in a number of other hydroids (for review of contraction pulse homologies, see Stokes & Rushforth, 1979). Extracellular recordings (Mackie, 1978) have demonstrated a fast spike-like event which immediately precedes a rapid all-or-none contraction, and which appears to arise from a more slowly conducted, long-lasting, depolarization. The present study describes intracellular recordings of these events, and investigates the ionic basis of their electrogenesis.

METHODS

Young colonies of *Agalma*, ranging in size from 1–10 cm, were obtained in considerable numbers during the month of April in plankton trawls at depths of 0–30 m in the Rade de Villefranche. Many fragments of adult colonies (which can reach several

metres in length) were also found. Complete small specimens could be maintained in the laboratory for several weeks if kept at 12 °C in fresh sea water. Intracellular recordings were made from gastrozooids either '*in situ*', by pinning down a whole colony, or by pinning down an individual after isolating it by cutting the stalk at the base joining it to the stolon. The conducting systems studied function essentially independently of electrical activity in the stolon (Mackie, 1978). Very fine Apuntia needles were used to pin down the preparation, to try to prevent damage in the fragile epithelium. The movements of the gastrozooids become particularly pronounced when they are restrained in any way, and are sometimes strong enough to tear the body wall and break free from the constraint. Intracellular recordings were made with 3 M-KCl-filled capillary glass microelectrodes, with tip resistances greater than 30 MΩ. The signal was amplified through a d.c. amplifier (20× amplification) and displayed on a Telequipment GM39 storage oscilloscope. The trace was photographed with a Polaroid CR-9 Land camera. Stimulating pulses (2–10 ms) were delivered from an isolated Digitimer stimulator triggered by a Farnell pulse generating system, either via two 25 μm platinum wires on the surface of the epithelium or via a polythene suction electrode placed on the base of the gastrozooid. This latter had the advantage of partially stabilizing and immobilizing the preparation. Nevertheless, because of the constant movements of the body wall, only transient (up to 5 m) recordings were obtained. Successive impalements were also difficult because of the toughness of the outer ectodermal membrane which frequently broke the microelectrode tip on penetration. The control solution used in the ionic experiments was fresh filtered seawater. Experimental solutions were based on an artificial sea water with a total salinity of 38% corresponding to sea water analyses carried out in the locality, which contained 516 mM-NaCl, 10·4 mM KCl, 11 mM-$CaCl_2$, 34 mM-$MgCl_2$ and 22 mM-$MgSO_4$. The solutions were buffered with 10 mM-$NaHCO_3$ at pH 8–8·2.

RESULTS

Intracellular recordings were obtained from all areas of the ectoderm, though stable penetration was easier near the base where the cells are thicker. The cells had resting potentials in the region of 40–70 mV. The movement of the tissues, however, caused a considerable amount of fluctuation in apparent membrane potential, much of which was probably artifact. The true resting potential was therefore difficult to determine with any precision.

Stimulation of fresh preparations, even if only just above threshold, usually fired a whole series of 'fast' action potentials (Fig. 2). Individual action potentials had durations of 20–40 ms, and overshoots ranging from a few mV to 20 mV. However the overshoot in any one burst sometimes remained constant, despite marked changes in the amplitude of the spikes (Fig. 2*b*). The fast action potentials were superimposed on a much slower depolarization. This had a duration of around 1 to 2 s (the downward or repolarization phase occupying 90% of this period) and the magnitude of the depolarization was close to the resting potential of the cell. The precise shape of the slow event was difficult to determine, owing to the presence of the 'fast' events. However, in one preparation (presumably damaged in some way) no contractions could be obtained, even with very large stimuli. In this preparation slow potentials,

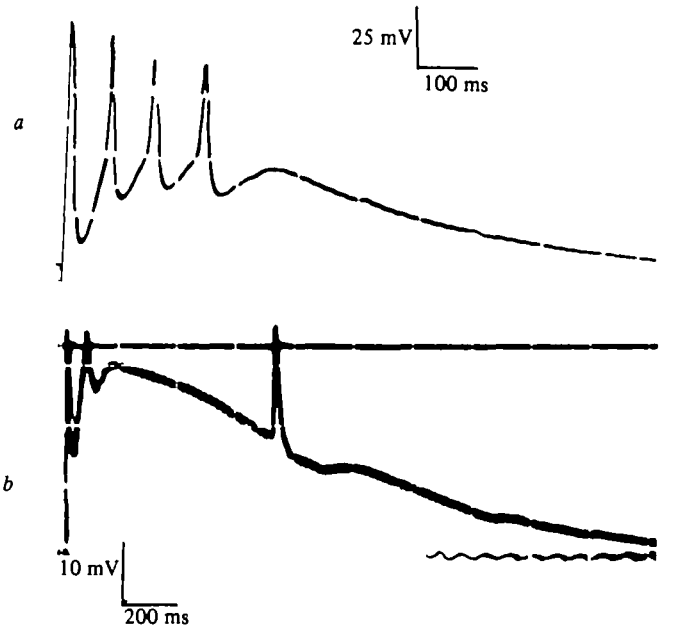

Fig. 2*a* and *b*. A series of action potentials triggered by a single stimulus. The fast action potentials are superimposed on a slower depolarisation.

Fig. 3. A slow potential which was elicited in one *Agalma* gastrozooid, which failed to show either rapid action potentials or contractions.

apparently very similar to those underlying natural contractile activity, were obtained (Fig. 3), which were free of superimposed fast potentials. The slow potentials in this case were not associated with any muscular activity. This finding, together with the constant shape of the slow potential in normal preparations, the constant overshoots of the superimposed action potentials, and results obtained in sodium-free solution (see below), all strongly suggest that the slow potentials are not movement artifacts, but represent a second real electrogenic phenomenon in the ectodermal cells. The slow potentials are graded events. They are much more labile than the fast pulses, fatigue rapidly, and disappear in older preparations. In these latter, repetitive activity disappears concurrently. Below the threshold of contraction, small (2–5 mV) potentials, with duration of only 200–300 msec, are obtained (Fig. 4), which increase rapidly in size and duration with increasing stimulus. The subthreshold events are presumably equivalent to the 'subthreshold' slow events recorded extracellularly by Mackie (1978). The precise relationship between the fast and slow components of

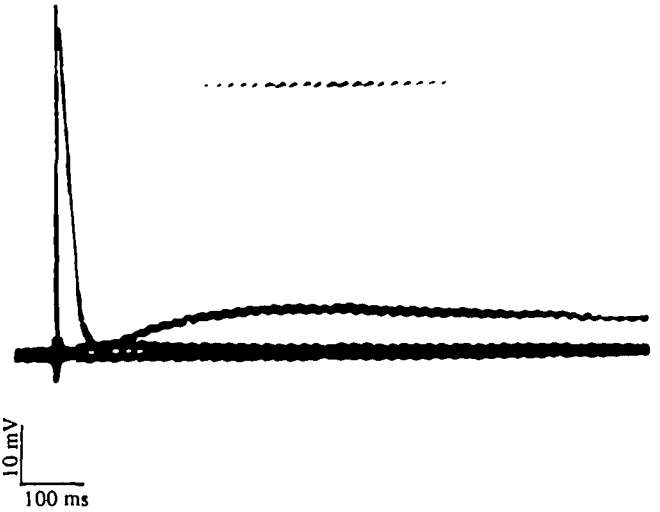

Fig. 4. Three sweeps at increasing stimulus strengths. The first stimulus did not evoke any electrical response. The second fired a slow potential, which appears as a small 'subthreshhold' event. The third fired a fast action potential which is followed by a larger and longer slow potential.

the response remains unclear. The first action potential of a series usually arrives before the slow potential. Its shape is also different from subsequent ones, with a simple one component rising phase (Fig. 2). It seems probable therefore, that this represents an action potential fired directly by the stimulus and subsequently conducted through the ectoderm. In some preparations bursts can be obtained in which this initial action potential is absent. The threshold to direct ectodermal stimulation is presumably marginally higher in these specimens. Subsequent APs in a burst appear to be triggered by a slower depolarizing 'prepotential'. Whether this prepotential is simply the underlying slow potential, and whether the latter triggers the fast action potentials remains unproven, though it would appear likely. The results of Mackie indicated that the two components had different conduction velocities suggesting that they are conducted in different systems. The highly variable duration, amplitude and rate of rise of the slow potential must, however, make precise measurements with extracellular recording techniques difficult.

IONIC EXPERIMENTS

In low sodium (all sodium chloride replaced by choline chloride sea-water), the contractions and fast action potential are rapidly abolished. The slow potentials continue for many minutes longer and are apparently identical to those obtained in normal sea water (Fig. 5). The graded nature of the response is particularly easy to demonstrate under these conditions. Ultimately, all electrical activity is abolished, although the slow writhing movements continue indefinitely. On return to normal sea water the action potentials recovers rapidly. Recovery of repetitive activity and the slow potential is much slower and more variable.

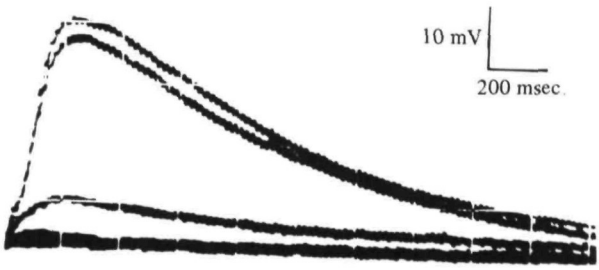

Fig. 5. Slow potentials in Agalma obtained after a few minutes in sodium-free solution. Four sweeps are triggered at successively higher stimulus strengths, to show the graded nature of the response.

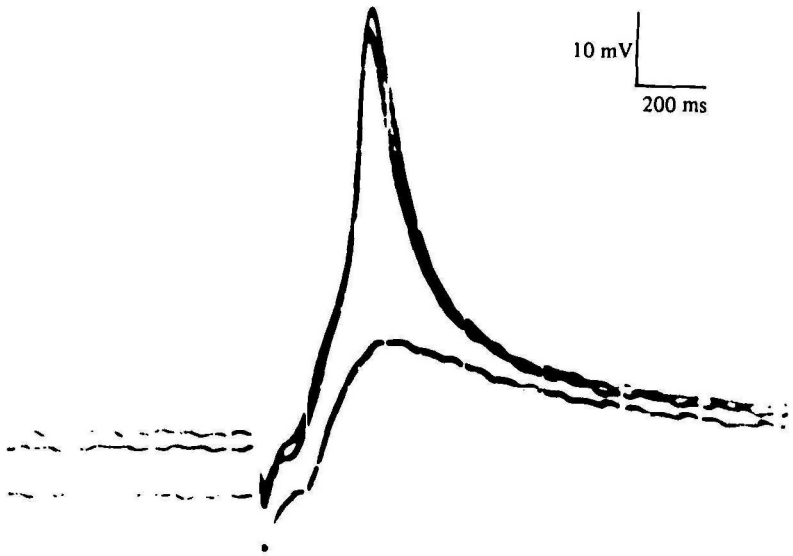

Fig. 6. Action potentials in sea-water containing 20 mM-manganese ions. The slow potential is entirely abolished.

Sea water with 20 mM manganese ions totally and rapidly blocks all movement of the gastrozooid. It also blocks the slow contractions and repetitive firing. Single APs of approximately normal amplitude can still be obtained (Fig. 6), though the stimulus threshold is very considerably increased. The rising phase of the action potential in 20 mM-Mn seems to be rather slower than in normal sea water: however, quantitative measurements are not available to substantiate this observation. All these effects are reversible. These results are rather difficult to reconcile with the observations of Mackie (1978), that both types of electrical responses were blocked by 1:5 Mg sea water. Attempts to repeat these observations indicated, on the contrary, that the fast pulses persist in this solution, though both slow responses and movements are completely blocked. It seems possible that the response had appeared to be blocked because of the increased threshold of stimulation in the presence of either high Mg or Mn. Low chloride sea water (NaCl substituted by $NaMeSO_4$) was without obvious effect on any of the responses.

DISCUSSION

The above results indicate that the fast action potentials are sodium-dependent in common with similar action potentials in two other siphonophore epithelia (B. M. Chain, G. Bone & P. A. V. Anderson, in preparation, Mackie, 1976). It is possible that a small calcium component is also present which may be involved in contraction-coupling in the ectoderm, though the source of calcium ions for contraction is unknown. The nature of the slow response is more problematical. A number of explanations are possible. The most likely seems to be that it represents some sort of excitatory junction potential which is conducted in the underlying nerve net. The response could be due either to a decrease or increase in the membrane permeability to one or more ions. In the former case, for example, the potential could be produced by a decreased potassium permeability. In the latter case (more usual) the inward current could be carried by calcium ions, but this seems unlikely since the response is not directly involved in initiating contraction. The most likely of all the possibilities is that the slow potential is produced by a synaptic slow Na current. Mg and Mn would therefore block the response by inhibiting synaptic transmission. The delay in the action of Na-free solution (compared with the immediate block of the fast potential) could be due to diffusional barriers surrounding the synaptic region of the cell membrane. Ultrastructural information on the structure of neuromuscular interactions in the epithelium would be helpful in elucidating these points.

As was noted in the *Introduction*, Siphonophore gastrozooids exhibit a number of striking similarities to Hyroid polyps. However, the results obtained above indicate that, though these parallels can be extended to the zooid contraction system, the behavioural response which has been the object of this study, they do not apply to the cellular mechanisms which are the basis of the response (see Macklin & Josephson, 1971; Chain, 1980). The gastrozooids seem to possess a 'dual' conduction system (the fast and the slow response), which is not paralleled in any of the Hydroid polyps studied so far. Furthermore, the presence of a sodium dependent action potential conducted *in* a muscular tissue, rather than by a parallel neural pathway, is not found in either of the two Hydroid conduction systems whose ionic properties have been studied. It is indeed a phenomenon which is almost wholly unknown outside the vertebrates, and its presence in a Siphonophore epithelium seems to reflect at a cellular level the high degree of sophistication and complexity which is such a characteristic feature of this whole order.

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