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EPITHELIAL CONDUCTION IN SALPS

I. PROPERTIES OF THE OUTER SKIN PULSE SYSTEM OF THE STOLON

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SUMMARY

1. The Outer Skin Pulse (OSP) system of the stolon of *Salpa fusiformis* was studied histologically and electrophysiologically.

2. The cells of the conducting epithelium are cuboidal, 4–10 μm in diameter and are connected by gap and tight junctions (Fig. 1). They have resting potentials of -75 to -96 mV.

3. Outer Skin Pulses are conducted as overshooting action potentials 84–104 mV in amplitude which are characterized by the absence of a hyperpolarizing undershoot during the repolarizing phase (Fig. 2A). When OSPs are evoked at frequencies in excess of 2 s^{-1} a pronounced plateau appears in the repolarizing phase (Fig. 2C).

4. Tetrodotoxin blocks OSPs in a manner which suggests that a sodium current is responsible for activation of the action potential.

5. Addition of 15 mM manganese to the bath blocks OSPs. This effect was initially accompanied by a reduction in OSP amplitude and an increase in duration (Fig. 5). This observation was interpreted as indicating the presence of a calcium influx during the action potential.

6. The results are compared with those obtained from epithelial conduction systems in other organisms, other embryonic tissues and excitable cells in other non-vertebrate chordates.

INTRODUCTION

Salps are free-swimming, pelagic tunicates which, because of their distribution and availability, have received little attention from physiologists. Recently, Mackie & Bone (1977) completed a series of electrophysiological studies of salps, and their results indicate that salps hold several attractions for physiologists, particularly for those interested in epithelial conduction.

An epithelial conduction system is a non-nervous, non-myoid conduction system which conducts action potentials. Epithelial conduction was first demonstrated in a coelenterate (Mackie, 1965) and has since been linked, albeit tentatively in many cases, with a variety of behaviours in several species (for review, see Spencer, 1974).

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The information available at present on the operation of epithelial conduction systems at their cellular level is extremely limited, largely because there is a scarcity of adequate intracellular records. This report describes intracellular recordings from epithelial cells in the stolon of *Salpa fusiformis* and provides information on the ionic basis of the action potential in this preparation.

The life-history, structure and development of salps has been well described (Berrill, 1950, and earlier reports cited therein). There are two stages in the life-cycle of salps; the blastozooid or colonial stage and the oozooid or solitary stage. The stolon is an ectodermal evagination of the oozooid which develops and buds to form chains of blastozooids (Fig. 1).

Oozooids produce stolons in discrete sections (Fig. 1) and while each section will be at a different developmental stage all the potential blastozooids within a given section will be similarly developed. Immature sections consist entirely of epithelial cells and as a result form stable preparations for intracellular recording.

MATERIALS AND METHODS

Oozooids of *Salpa fusiformis* were collected from the plankton in Villefranche Bay, France, during the months of March and April 1977 and were maintained at the Station Zoologique in a 14 °C cold room in large bowls of sea water. Under these conditions, stolons of sufficient size for handling could be removed from the oozooids after 24–48 h.

The stolons were transferred to a Sylgard lined preparation dish and held in position by two fine polyethylene suction electrodes which also served as an extracellular recording electrode and a stimulating electrode. All recordings were done on the most immature sections of a stolon and the suction electrodes were positioned so as to span a portion of that section 0·5–1·0 cm in length. Intracellular recordings from cells located between the two suction electrodes were obtained with 3 M-KCl filled 40–50 MΩ microelectrodes and the resulting signals were displayed on a Tektronix 5103N Storage Oscilloscope. Events recorded by the extracellular electrode were passed directly to the amplifiers of the oscilloscope. Stimuli were supplied by a Grass S48 stimulator. All experiments were conducted at room temperature (20–21 °C).

Tissue for electron microscopy was fixed in cacodylate buffered 5% glutaraldehyde in sea water and post-fixed in buffered 1% osmium. Thin sections were stained in uranyl acetate and lead citrate and examined in a Philips EM 300 electron microscope.

RESULTS

Ultrastructure of the conducting epithelium

The structure and development of the salp stolon have been described elsewhere (Berrill, 1950; Toselli & Harbison, 1977), so this report will concentrate on ultrastructural aspects of the stolon relevant to the subject of epithelial conduction. Action potentials were commonly recorded from cells on the surface of the stolon. These cells are cuboidal and have a diameter of 4–10 μm (Fig. 1 B). Each has a large nucleus which occupies approximately 40% of the cell volume. Gap junctions are commonly found

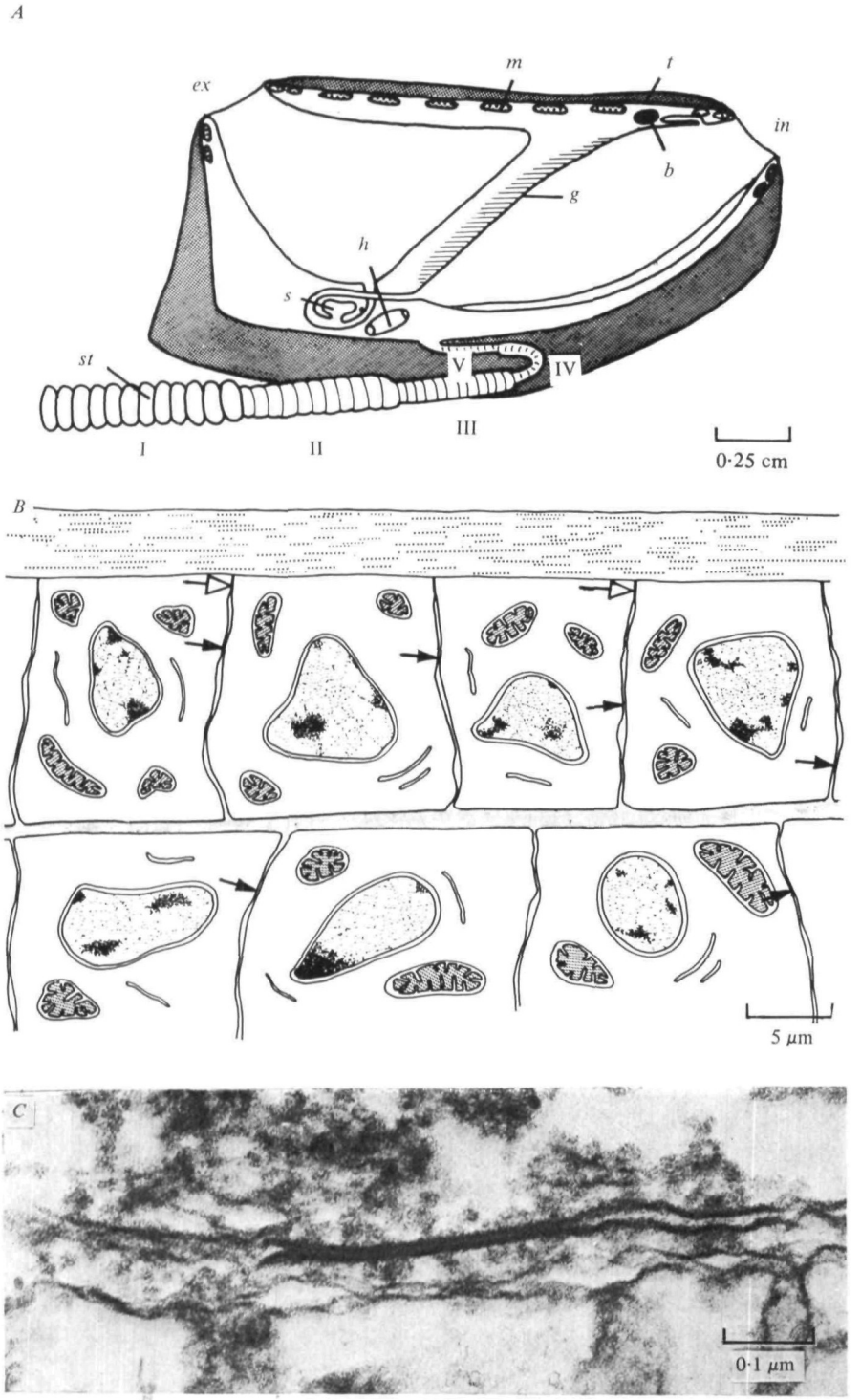

Fig. 1. (A) A simplified drawing of an oozooid cut in sagittal section to reveal the location and form of the various internal structures. A stolon (*st*) composed of five discrete sections (I–V) is shown. *in*, inhalent aperture; *ex*, exhalent aperture; *m*, cut end of muscle band; *t*, tunic; *b*, brain; *h*, heart; *s*, stomach. (B) Diagrammatic representation of a section through the outer surface of a newly formed section of stolon (IV or V in Fig. 1A). The epithelial cells are cuboidal and connected by gap junctions (small arrows) and tight junctions (large arrows) at their outer edges. *t*, tunic. (C) High power electron micrograph of a gap junction between two epithelial cells in a stolon.

between the cells (Fig. 1 C) and what appear to be tight junctions are invariably found at the outer margin of the cells. Nerves were never observed in any stolon of the developmental stages used for recording.

Recordings

Extracellular recordings indicate that the stolon contains only one conduction system. The stolon is normally electrically quiet, but a single electrical stimulus to any part of it evokes a single impulse, 1–2 mV in amplitude (Fig. 2A), which is conducted at a rate which depends on the developmental stage of each section. For instance, in one stolon composed of two sections the older more mature section conducted impulses at 4·6 cm s^{-1} while the less mature section conducted at 7·6 cm s^{-1}. A similar observation was made by Mackie & Bone (1977). The absolute refractory period of this conduction system is 7 ms although in this instance no attempt was made to relate this value to the developmental stage of the particular section.

Mackie & Bone (1977) described four separate 'skin pulse' systems and found that only one of these, the Outer Skin Pulse (OSP) system, extended into the stolon. Since

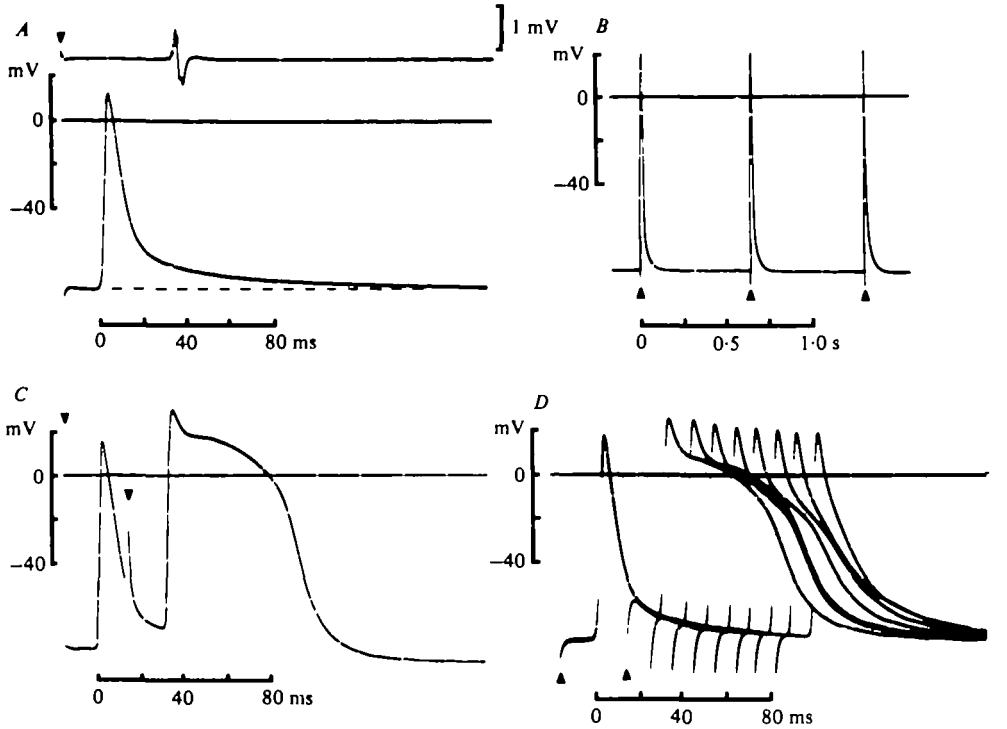

Fig. 2. (A) A single action potential recorded extracellularly (upper trace) and intracellularly (lower trace) from the Outer Skin Pulse (OSP) system of *S. fusiformis*. In this, and all subsequent figures, the stimulus artifact is marked by a dark triangle. (B) An intracellular record of three action potentials evoked by low frequency stimuli. Note the similar waveform of each. (C) Intracellular record of two action potentials evoked at an interstimulus interval of 33 ms. Note the prominent plateau and increased amplitude of the second action potential. (D) A superimposed intracellular record of eight pairs of OSPs. The interstimulus interval was increased by 10 ms for each pair and while the first action potential in each pair superimposed the second appeared successively later.

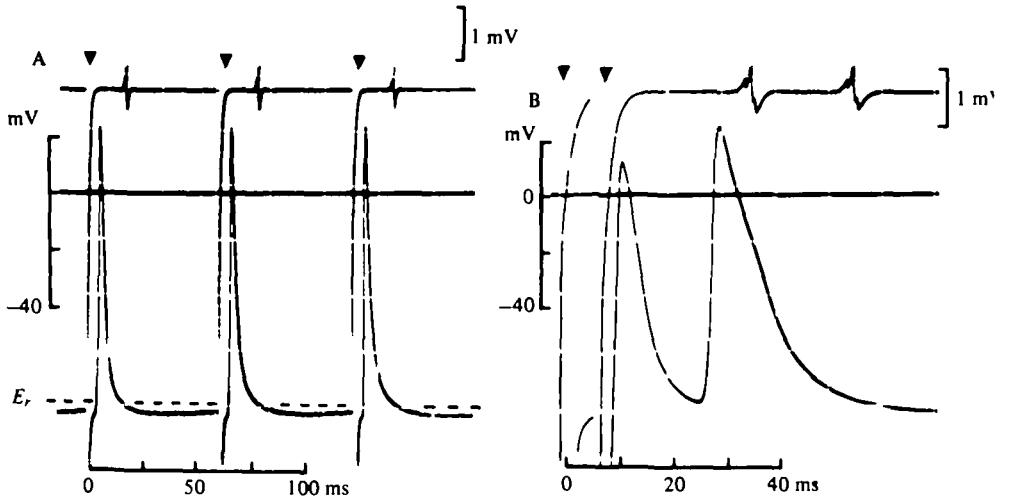

Fig. 3. Records of OSPs recorded from one unusual stolon. (A) Three low frequency action potentials illustrating the small hyperpolarizing undershoot typical of OSPs in this stolon. The dotted line, marked E_r, indicates the normal resting potential of the cell. (B) Two OSPs evoked 17 ms apart. Note the absence of the plateau normally present in closely spaced action potentials.

this observation was confirmed in this study, impulses recorded from the stolon will hereafter be referred to as Outer Skin Pulses.

Intracellular recordings were routinely made from cells on the outer surface of the stolon. These cells could be impaled relatively easily, and resting potentials in the range −75 to −96 mV could be maintained for periods well in excess of half an hour. No spontaneous electrical events were ever recorded from these cells. However, they do propagate OSPs, which, when recorded intracellularly, have a waveform that is characterized by the absence of any hyperpolarizing undershoot during the repolarizing phase of the action potential.

A single electrical stimulus to any part of the stolon evokes an over-shooting action potential with a peak amplitude of 84–104 mV (Fig. 2A). The peak is reached 4 ms after the start of the action potential. The amplitude falls to half its maximum value after an additional 7 ms and then repolarization slows up, taking a further 110 ms for the membrane potential to return to its pre-spike level. With repetitive stimulation at frequencies lower than 2 Hz, the evoked action potentials all appear identical (Fig. 2B). However, if the stimulating frequency is increased, the waveform changes. While the slope of the rising phase remains unchanged, the amplitude of the spike increases and a plateau appears during the repolarizing phase (Fig. 2C). The magnitude of the plateau and the amplitude of the action potential were greatest at shorter inter-stimulus intervals (Fig. 2D).

The action potentials described above were typical of those recorded from the majority of stolons. However, one stolon consistently produced action potentials which contained a very slight hyperpolarizing undershoot (approx. 4 mV in amplitude) (Fig. 3A). Furthermore, when evoked repetitively these action potentials did not form such noticeable plateaus and only showed small amplitude increases (Fig. 3B). The stolon

which produced these action potentials was removed from a large oozooid, 5 cm in length. These action potentials closely resemble those recorded from the mature OSP system of *S. fusiformis* blastozooids (Anderson, Bone, Mackie & Singla, 1979).

Effect of ions and drugs

The OSP system is reversibly blocked by tetrodotoxin. Fig. 4A illustrates the changes effected by the addition of TTX to the preparation bath. Sufficient TTX was added to give a final concentration of 1×10^{-5} g/ml and the stolon was stimulated once every 100 s. OSPs, here recorded extracellularly, were blocked after approximately 40 min. Prior to the breakdown in conduction, the conduction velocity fell from 8·0 to 4·3 cm/s and the waveform of the OSP changed, with the amplitude of the spike decreasing and the positive going phase of the extracellular spike becoming progressively longer. The stolon used in this experiment resumed normal propagation of evoked OSPs after 3 h in sea water.

Intracellular records from another preparation indicate that TTX affects the rising phase of the action potential by reducing both the rate of depolarization of the spike and its amplitude (Fig. 4B). As the spike illustrated in Figure 4B disappeared it was replaced by a slow depolarization of the baseline. The amplitude of this depolarization was dependent on the stimulus intensity (Fig. 4C) and was partially reduced by the addition of manganese ions. In this experiment, the stimulating electrode was approximately 1–2 mm from the recording site. The effect of TTX on pairs and trains of spikes was not tested.

Manganese added to the normal sea water of the preparation bath reversibly blocked

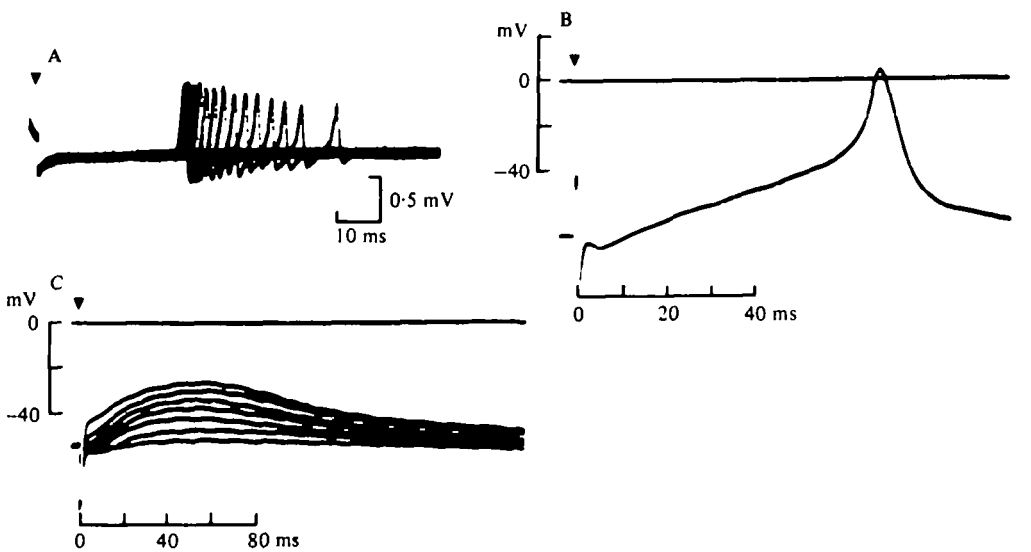

Fig. 4. Records of the effect of tetrodotoxin on the OSP system. (A) An extracellular record of some 24 action potentials recorded in the presence of 1×10^{-5} g/ml TTX. The action potentials were evoked at 100 s intervals and conduction was blocked after approximately 40 min. (B) A single OSP recorded intracellularly in the presence of 1×10^{-5} g/ml TTX. (C) Superimposed intracellular records of the evoked slow depolarization that remained after the spike had been abolished by TTX. Each curve was evoked at a successively greater stimulus intensity.

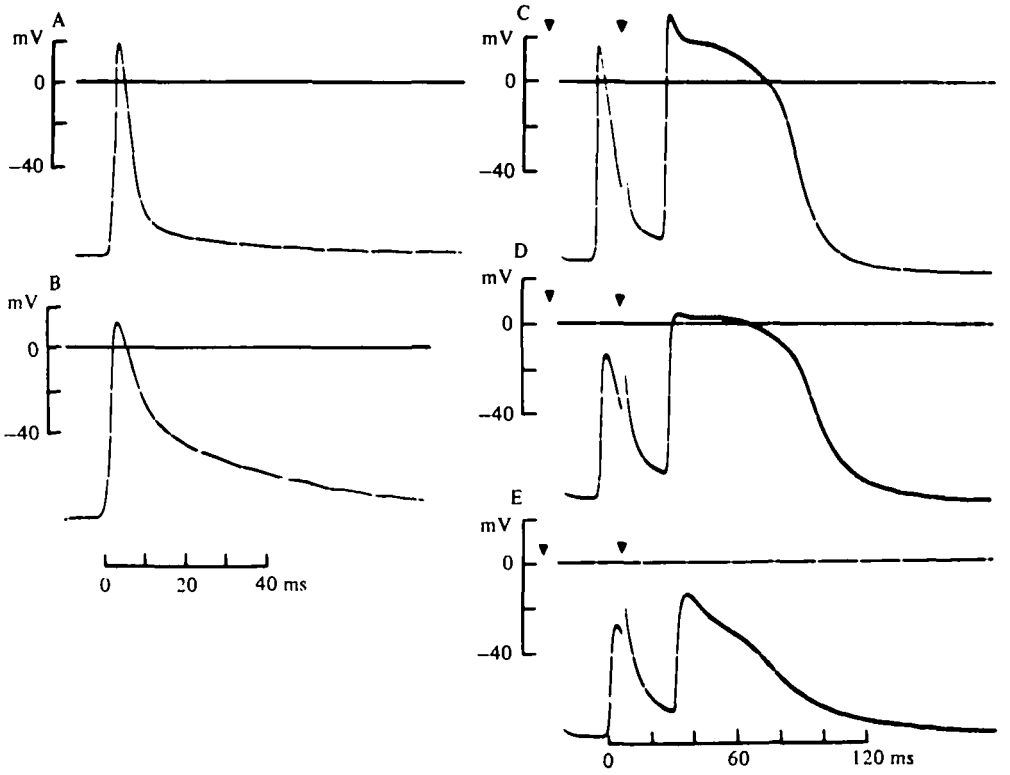

Fig. 5. Intracellular records of the effect of manganese on OSPs. (A) A single action potential recorded in sea water before addition of manganese. (B) The same after addition of the manganese. Note that the amplitude of the OSP has diminished and the repolarizing phase has been prolonged. (C, D, E) Intracellular records of a pair of closely separated (30 ms) OSPs evoked before (A), 16 min (B) and 28 min (C) after the addition of manganese.

conduction of OSPs. This block sometimes occurred in the absence of any noticeable change in the intracellular records until that moment when the OSPs disappeared. However, if care were taken to ensure that the stimulating electrode was positioned in the centre of a section and intracellular recordings made near a cut end of the same section, progressive changes in the waveform of the impulses could be observed. The effect of manganese was typically to reduce the amplitude of the action potentials and to increase their duration by reducing the rate of repolarization (Fig. 5B). These effects were very rapid, sometimes occurring within 3 min of the addition of sufficient $MnCl_2.4H_2O$ for a 15 mM solution. Manganese similarly affected the action potentials evoked by closely spaced stimuli (Fig. 5C–E).

DISCUSSION

Epithelial conduction systems have been recognized for over 10 years, and while relatively little is known about spike production and propagation at the cellular level, considerable information about the morphological organization and ultrastructure of epithelial conduction systems has been accumulated. The cells that comprise the various identified conducting epithelia are typically small (10–30 μm in diameter) and gap junctions are invariably found between the cells (Roberts & Stirling, 1971; Mackie & Singla, 1975; Mackie, 1976). These are also features of the Outer Skin Pulse system of the salp stolon (Fig. 1 B, C).

With the exception of the results presented here, intracellular recordings have been obtained from only four epithelial conduction systems; the skin of amphibian tadpoles (for review, see Roberts, 1975); the skin of tadpole larvae of the ascidian *Dendrodoa* (Mackie & Bone, 1976); the *rete mirabilis* cell of the siphonophore *Hippopodius* (Mackie, 1976) and the exumbrellar ectoderm of *Euphysa*, a hydromedusan jellyfish (R. K. Josephson & W. E. Schwab, in preparation). The results described here compare most closely with those obtained from amphibian tadpoles.

For a limited period of its development (Nieuwkoop and Faber stages 24–40) the skin of *Xenopus* is capable of conducting overshooting action potentials. These impulses, and those of *Dendrodoa*, are long duration events which contain a characteristic plateau during the repolarizing phase. In the *S. fusiformis* stolon (another embryonic tissue), the action potentials evoked by low frequency stimuli do not contain a plateau. They do, however, have long durations which, for the most part, consist of a slow repolarization. This slow repolarization is not a feature of OSPs recorded from the mature outer skin pulse system of blastozooids (Anderson *et al.*, 1979) or of OSPs recorded from one stolon (Fig. 4A). Furthermore, when they are evoked repetitively the action potentials recorded from these epithelia do not contain a plateau (Fig. 3B and Anderson *et al.*, 1979) as do the action potentials typically recorded from stolons (Fig. 2C, D). This relationship between the long duration of the first action potential and the tendency for subsequent action potentials evoked at high frequency to contain a plateau suggests that the slow repolarization, in fact, represents a small plateau which becomes more pronounced with repetitive stimulation.

The magnitude of this plateau increases with increasing stimulus frequency (Fig. 2D). This relationship suggests either that: (1) the currents responsible for the plateau are facilitated by closely spaced stimuli; (2) currents responsible for repolarization inactivate and remain partially inactivated when action potentials follow in close sequence; or, (3) currents which are normally inactivated by the repolarization mechanisms of the cell are potentiated when action potentials follow in close sequence. In view of the embryonic nature of the tissue and the fact that the plateau disappears as the stolon matures, one or both of the latter two possibilities is perhaps the more likely. Action potentials recorded from developing tunicate tadpole cells (Takahashi, Miyazaki & Kidokoro, 1971) are similar to those of the OSP system inasmuch as they contain a plateau which disappears as the cells mature. On the basis of changes in the current/voltage relationship of the muscle cells at different stages of their development, Takahashi *et al.* (1971) suggested that the disappearance of the plateau may be the result of the establishment of a mechanism such as delayed rectification.

If a mechanism such as delayed rectification were present but poorly developed in immature stolon cells, action potentials recorded from those cells could have a small plateau which, when the action potentials are evoked in close sequence, might increase in magnitude. As the cells mature and the rectification mechanism becomes more efficient, the plateau might then disappear. The absence of any significant plateau in OSPs recorded from mature cells (Anderson *et al.*, in press) supports this.

The experiments with TTX (Fig. 4A, B) indicated that a sodium current is responsible for the depolarizing phase of the action potential. In other excitable cells, TTX specifically blocks sodium currents. The reduction in amplitude and the change in the slope of the rising phase of the action potential recorded in the presence of TTX are consistent with a similar action of this drug on the stolon cells.

OSPs are also blocked by manganese, a known calcium antagonist. It is possible that the decrease in amplitude and increase in duration of OSP recorded prior to this breakdown in conduction may be the result of the elimination by manganese of an inward calcium current and a calcium activated repolarization mechanism similar to that present in *Helix* neurones (Meech, 1974).

The calcium current necessary for this mechanism may be responsible for the slow depolarization that remained after the action potential had been eliminated by TTX. If this depolarization were indeed produced by a calcium current it would, by necessity, have to flow through TTX-insensitive calcium channels similar to those present in squid axon (Baker, Hodgkin & Ridgway, 1971). The evidence in support of the suggestion that this depolarization is due to a calcium influx is as follows. The stimulating site in this experiment was only 1–2 mm from the recording site, so any non-regenerative events evoked by the stimuli might well reach the recording site. Furthermore, the amplitude of the depolarization was proportional to the stimulus intensity and was slightly reduced by the addition of manganese to the bath. Alternatively, this depolarization might be a stimulus artifact produced by the unusually close proximity of the recording and stimulating electrodes.

The suggested presence of a calcium activated repolarization mechanism does not exclude the possibility that other repolarizing mechanisms may also be present. In *Helix* neurones, both a calcium activated and a voltage activated potassium current serve to repolarize the cell (Meech & Standen, 1975).

The times taken for the various drugs used here to have their observed effect may seem somewhat excessive but they are probably not truly representative of the actual effect times. The extremely small size of the impaled cells required that the drugs be added at the edge of the preparation bath, to prevent dislodgement of the electrode. Consequently, the times measured in these experiments include a diffusion interval and in the case of the manganese experiments, a dissolving time. Furthermore, the presence of tight junctions throughout the surface epithelium will probably make the preparation relatively impermeable. If these various factors are taken into consideration, the actual times involved become more realistic.

In other non-vertebrate chordate excitable cells, i.e. *Amphioxus* muscle (Hagiwara & Kidokoro, 1971) and tunicate eggs (Miyazaki, Takahashi & Tsuda, 1974) a sodium/calcium current is also responsible for the action potential. A combined sodium/calcium current has not, however, been shown to be a general feature of epithelial conduction systems. In amphibian tadpoles, the skin pulse is due to a TTX-sensitive sodium current but the contribution of calcium is very small (Roberts & Stirling, 1971).

Likewise, in the *rete mirabilis* cells of *Hippopodius*, the action potential is a sodium event (Mackie, 1976) although, in the latter case, a careful study to determine whether there was any contribution from calcium was not made.

Despite this slight variation between the amphibian skin pulse system and the stolon OSP system, the two systems are similar in most respects. The action potentials from each contain a plateau, at least in the immature OSP system, and are both TTX-sensitive events. The greatest similarity between the two may be the fact that both are embryonic tissues. The presence of a plateau on action potentials recorded from amphibian tadpole skin, from developing tunicate muscle (Takahashi *et al.* 1971), and from a mammalian heart (Couch, West & Hoff, 1969) led Roberts to suggest (1975) that action potentials with a plateau may be a consistent feature of embryonic excitable cells. The results presented here certainly support this view and give further insights into the changes that occur as the cells mature.

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