J. exp. Biol. (1979), **82**, 227–238
With 6 figures
Printed in Great Britain

Mn^{2+} IONS PASS THROUGH Ca^{2+} CHANNELS IN MYOEPITHELIAL CELLS

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(*Received* 25 *September* 1978)

SUMMARY

1. Intracellular recordings were made from the myoepithelial cells of the proventriculus of the marine polychaete worm *Syllis spongiphila*. Overshooting responses were elicited either by carbamylcholine added to the bathing medium or by directly applied intracellular current pulses.

2. In control artificial sea water (ASW) directly applied current pulses elicited regenerative responses of 68–119 mV in amplitude and 70–1800 ms in duration; these responses were associated with contractions of the myoepithelial cells.

3. Both pharmacologically and electrically elicited responses were reversibly abolished in Ca-free ASW and were unaffected by TTX or low-sodium solutions. Regenerative responses were elicited by direct intracellular stimulation in calcium-free ASW containing 1 mM-Ba^{2+} or 10 mM-Sr^{2+}. Directly elicited responses were blocked reversibly in ASW containing calcium and 15–20 mM-Co^{2+} or 2·5–10 mM-Ni^{2+}; they were blocked irreversibly in ASW containing calcium and 10 mM-La^{3+} or 100 μM-Zn^{2+}.

4. Regenerative responses were elicited in Ca-free solutions containing 10–50 mM-Mn^{2+}; these responses were not associated with contractions, were consistently of longer duration than responses elicited in control ASW, and were blocked by 20 mM-Co^{2+} or 10 mM-La^{3+}. The overshoots of Mn^{2+} responses elicited in both Na-free and Na-containing, Ca-free solutions increased as the external concentration of Mn^{2+} was increased, with a slope of about 27 mV per 10-fold change in concentration of Mn^{2+}. In Ca-containing solutions the slope was reduced to about 15 mV per 10-fold change.

5. The results indicate that the myoepithelial cells generate Ca-spikes and that Mn^{2+} ions, in addition to Sr^{2+} and Ba^{2+} ions, pass through the Ca^{2+} channels of the myoepithelial cell membranes. Although Mn^{2+} can replace Ca^{2+} in generating spikes, it apparently cannot replace Ca^{2+} in initiating contraction, and it many compete with Ca^{2+} in activating repolarization of the cell.

INTRODUCTION

The action potentials generated by a variety of excitable cells are known to be calcium-dependent (Hagiwara, 1973; Reuter, 1973). In several different preparations, Sr^{2+} and Ba^{2+} ions replace calcium ions (Fatt & Ginsberg, 1958; Hagiwara & Naka,

1964; Kerkut & Gardner, 1967; Fukuda & Kawa, 1977) while Mn^{2+}, Co^{2+} and La^{3+} ions inhibit calcium influx (Hagiwara & Nakajima, 1966; Hagiwara & Takahashi, 1967; Kerkut & Gardner, 1967).

Recently, investigators have found that Mn^{2+} ions, previously shown only to block calcium events, apparently pass through calcium channels in certain preparations. Ochi (1975) demonstrated in guinea-pig papillary muscle a slow inward current that was Mn^{2+}-dependent and TTX-resistant, and Fukuda & Kawa (1977) showed that Mn^{2+} ions (as well as several other ions) could replace Ca^{2+} ions in the generation of action potentials in muscle fibers of the larvae of the beetle *Xylotrupes dichotomus*. Further, Hagiwara & Miyazaki (1977) showed a small inward Mn^{2+} current, presumably through calcium channels, in voltage-clamped starfish egg cells. These observations are of comparative value in contributing to a general understanding of the permeability of membranes to calcium.

The experiments described in this paper extend the analysis of calcium permeability to a different preparation, the proventriculus of the marine polychaete worm *Syllis spongiphila*. The results show that the myoepithelial cells of the proventriculus are capable of undergoing regenerative activity, that the overshooting spikes elicited both by transmitter substance applied to the bath and by directly applied intracellular current pulses are calcium-dependent, and that Mn^{2+} (in addition to Sr^{2+} and Ba^{2+}) ions can replace Ca^{2+} ions in generating spikes, apparently by passing through calcium channels. The results obtained using some other polyvalent cations are also included. An abstract of some of the results described here has been previously published Anderson, 1977).

METHODS

Specimens of the marine polychaete worm *Syllis spongiphila* were collected in the Harbor of San Juan, Puerto Rico. Animals were kept in sea water in a constant-temperature chamber at 20 °C. For experiments, the proventriculus of an animal was dissected free and pinned to the bottom of a small (0·35 ml volume), Sylgard-lined plexiglass chamber filled with artificial sea water (ASW). A detailed description of the preparation was published previously (Anderson & del Castillo, 1976).

Depolarizing responses were elicited from the myoepithelial cells of the proventriculus either pharmacologically, by the addition of carbamylcholine to the bath (Anderson & Mrose, 1978), or electrically, by means of directly applied current pulses. In both types of experiments a glass, 3 M-KCl-filled recording microelectrode (5–15 MΩ resistance) was connected to the oscilloscope via a high-input impedance preamplifier. An agar bridge connected the bath to ground through a calomel cell. To elicit responses with directly applied current pulses, a second microelectrode was placed either in the same cell as the recording electrode, or, since the cells are electrically coupled (Anderson & del Castillo, 1976), in an adjacent cell. The stimulating electrode was connected to the output of a Grass SD-9 stimulator (early experiments) or a WPI Model 301 Anapulse stimulator (later experiments). The current applied was monitored on a second trace of the oscilloscope by recording the voltage drop across a 10 kΩ resistor between the calomel cell and ground. Oscilloscope recordings were photographed on film with a kymograph camera. All experiments were performed at room temperature.

The control ASW used in most experiments had the following ionic composition (mM): Na, 457; K, 9·7; Ca, 10·1; Mg, 52·5; Cl, 534; HCO_3, 2·5; SO_4, 27·7 (Welsh, Smith & Kammer, 1968). The pH was adjusted to 7·5–7·6 with dilute HCl. Ca-free ASW was made by replacing Ca^{2+} by an equimolar amount of Mg^{2+}. Solutions containing Mn^{2+} (except Na-free solutions) were prepared in one of two ways: (1) the total concentration of divalent cations of the control ASW was kept constant by replacing Mg^{2+} ions with Mn^{2+} ions, or (2) the $[Mg]_o$ was kept constant and Mn^{2+} was added up to 50 mM. The hypertonicity of the latter solution did not appear to affect the preparations adversely; resting potentials remained within the normal range, and the results obtained did not differ significantly from the results obtained from solutions containing a constant concentration of divalent cations.

The other polyvalent cations tested were added as chlorides. Co^{2+} and Zn^{2+} were added to the control ASW; Mg^{2+} at high concentrations replaced Na^+ in a ratio of 2 Mg^{2+} ions for 3 Na^+ ions; Ba^{2+}, Sr^{2+} and La^{3+} were added to ASW in which HCO_3^- and SO_4^{2-} anions were replaced by Cl^- (NaCl, 454 mM). Solutions containing tetraethylammonium chloride (TEA) were made by replacing Na^+ ions with an equivalent number of TEA^+ ions. Tetrodotoxin (TTX, Calbiochem) was added to control ASW; the solutions used were shown to inhibit spontaneous activity recorded from the third roots of abdominal ganglia of crayfish. EGTA [Ethylenebis(oxyethylenenitrilo)] tetraacetic Acid (Eastman) was added to Ca-free ASW. Stock solutions of Carbamylcholine Chloride (Carb, Sigma) were made using distilled water and diluted with ASW to the appropriate concentration shortly before use.

In low-Na and Na-free solutions, sucrose was used to replace Na^+ in a ratio of 1·83 sucrose to one Na^+. Sucrose was used instead of Tris or choline because experiments in which Na^+ was replaced by Tris (tris(hydroxymethyl)methylamine) resulted in a complete loss of membrane potential. The Tris could have acted to depolarize the cells by possibly binding to the ACh receptor (Wilson, Clark & Pellmar, 1977), to increase the permeability of the membrane to calcium or even to Tris (Maeno, Edwards & Anraku, 1977), or it could have exerted a toxic effect (Gillespie & McKnight, 1976). Replacing Na^+ with choline elicited a depolarizing response and rendered the membrane non-responsive to both indirect and pharmacological stimulation; choline may mimic the excitatory receptor in the *Syllis* proventriculus and block the transmitter receptor sites (Hutter, 1952).

The pH of all solutions was brought to 7·3–7·6 by the addition of dilute HCl. Solutions containing Zn^{2+} and La^{3+} were used at pH 6·75.

Bathing solutions were changed by drawing off the original solution and washing the chamber thoroughly (at least five times) with the new solution.

To correlate the intracellularly recorded electrical activity with mechanical activity elicited by direct stimulation, light micrographs of the proventriculus were taken under various experimental conditions. The proventriculus was positioned so that the slit-like lumen around which the myoepithelial cells are radially arranged (Smith, del Castillo & Anderson, 1973) was orientated vertically. The microscope was focused on the longest myoepithelial cells, which flank the two sides of the lumen, and the proventriculus was illuminated from the side and/or below, to emphasize the contrast between the lumen and the myoepithelial cells. A circuit from the shutter contact of the camera provided a signal on a trace of the oscilloscope that permitted correlating the

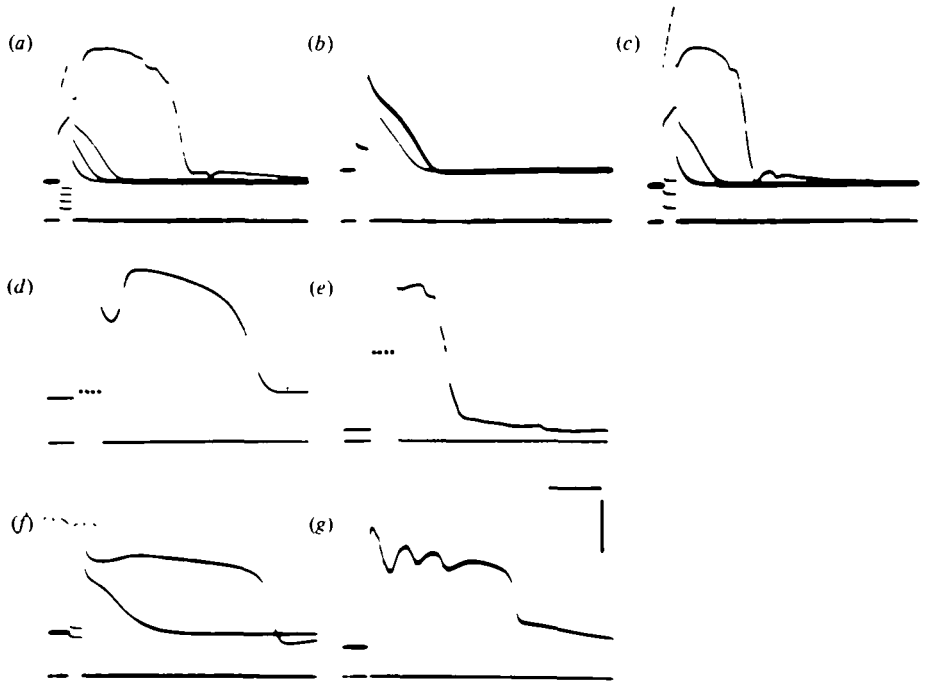

Fig. 1. Intracellularly recorded responses (top traces) elicited by directly applied current pulses (bottom traces) under various experimental conditions. See text for details. (*a–c*) Responses were reversibly abolished in Ca-free ASW; records were taken from the same cell in control ASW (*a*), after 6 min in Ca-free ASW (*b*), and 2 min after return to control ASW (*c*). (*d*, *e*) Responses were unaffected by the replacement of 95 % of the external Na^+ by sucrose (*d*) or by 10^{-8} M TTX (*e*); records were taken from different preparations (dotted lines indicate peaks of current pulses). (*f*, *g*) Regenerative responses were supported by 1 mM-Ba^{2+} (*f*) or 10 mM-Sr^{2+} (*g*) in Ca-free ASW. Calibrations, 40 mV; 2×10^{-6} A; 400 ms (*a–f*) and 2 s (*g*).

electrical activity recorded during the time the shutter was open and the mechanical condition of the proventriculus as photographed at that time.

RESULTS

Regenerative responses. Responses recorded intracellularly from myoepithelial cells during spontaneous activity (del Castillo, Anderson & Smith, 1972) or during activity initiated by indirect stimulation (Anderson & del Castillo, 1976) typically exhibit a series of excitatory junctional potentials that sum to a peak which overshoots the zero reference level. Overshooting depolarizations may also be elicited by acetylcholine (ACh) or carbamylcholine (Carb) applied to the bath; the rapidly rising, overshooting event is followed by a prolonged plateau of depolarization (Anderson & Mrose, 1978). Experiments using directly applied current pulses showed that the overshooting responses are regenerative events and not simply the sums of junctional potentials. In these experiments a current-passing microelectrode was placed within the same cell as the recording electrode or in an adjacent cell. Because the myoepithelial cells are closely coupled electrically (Anderson & del Castillo, 1976), large current pulses ($0{\cdot}7$–$4{\cdot}0 \times 10^{-6}$ A and 50–200 ms duration) were required to elicit responses. Fig. 1(*a*) shows an example of an overshooting depolarization elicited in control ASW

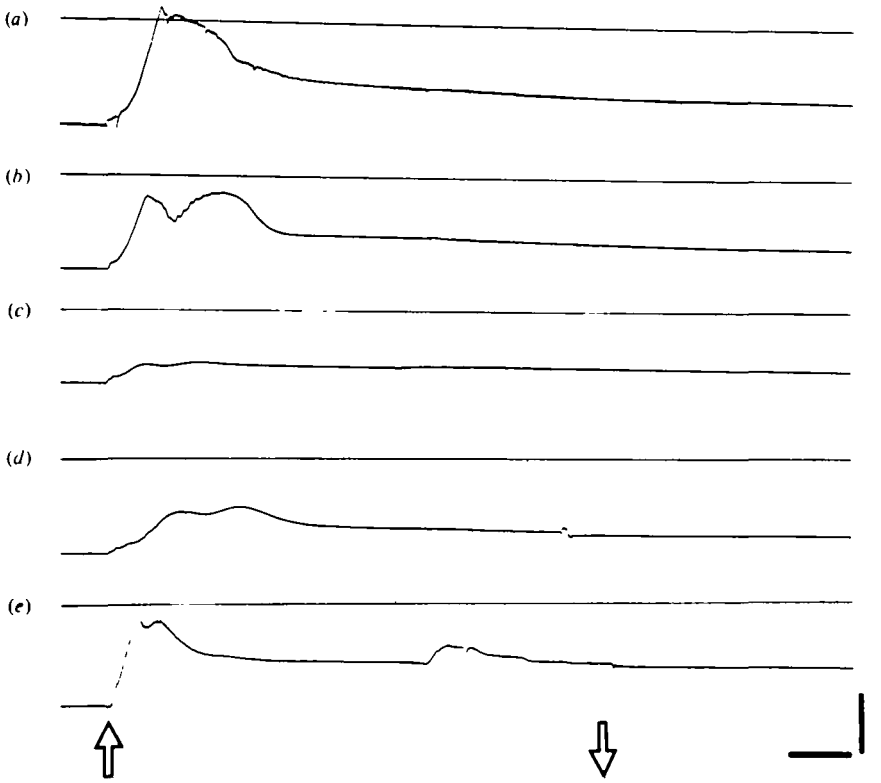

Fig. 2. Reduction in amplitude of intracellularly recorded responses elicited by the addition of Carb to the bath in Ca-free ASW containing 1 mM EGTA. Top traces, zero reference level; bottom traces, intracellular activity. (*a*) Control ASW; (*b*, *c*) after 3 and 30 min, respectively, in Ca-free ASW containing EGTA; (*d*, *e*) after 8 and 18 min, respectively, upon return to control ASW. Arrows indicate application and washing out of Carb. Calibrations, 40 mV; 4 s.

subthreshold pulses elicited simple electrotonic responses. Responses elicited in control ASW ranged from 70 to 1800 ms in duration and 68 to 119 mV in amplitude (24 preparations). These responses were associated with contractions of the myoepithelial cells.

Calcium-dependence of regenerative responses. To test the ionic dependence of the regenerative responses, stimuli were applied to preparations bathed in low-sodium, sodium-free and calcium-free solutions. In solutions in which all or 90 % of the sodium was replaced by sucrose, the application of Carb to the bath elicited both overshooting and nonovershooting responses. Overshooting responses to directly applied current pulses were consistently elicited in ASW in which 95 % of the sodium was replaced by sucrose. An example of such a response is shown in Fig. 1(*d*). These observations suggest that sodium ions do not contribute significantly to the regenerative event. This conclusion is further supported by the observation that responses were elicited both pharmacologically and electrically in the presence of 10^{-6} and 10^{-5} TTX. Fig. 1(*e*) illustrates a response elicited by direct stimulation after the preparation was bathed for 9·5 min in 10^{-5} M TTX.

The application of Carb to the bathing media of preparations exposed for many

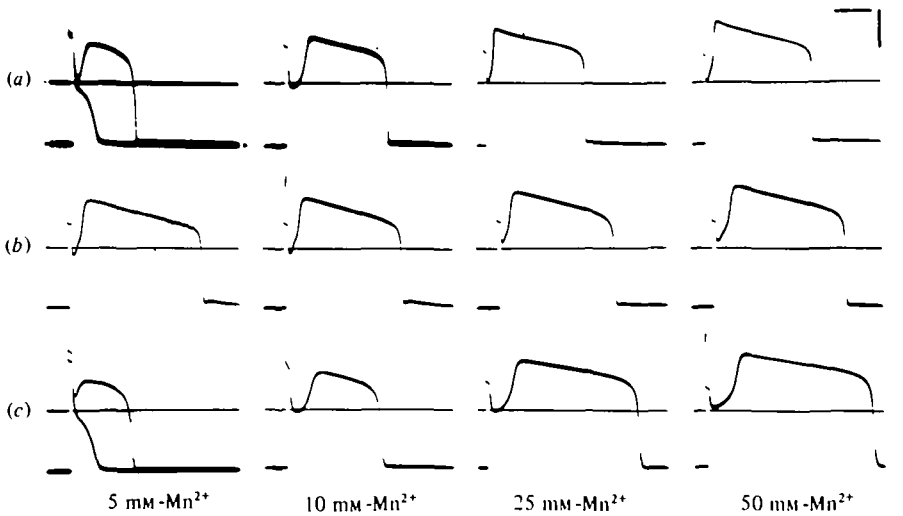

Fig. 3. Effects of Mn^{2+} in the bathing medium. Directly elicited responses (bottom traces) were recorded intracellularly from a single cell when the preparation was bathed in solutions containing 5–50 mM-Mn^{2+}. (*a*) Ca-free ASW; (*b*) Ca-containing (10 mM) ASW; and (*c*) Na-free (replacement by sucrose), Ca-free ASW; all three sets of solutions contained a constant concentration of Mg^{2+} ions. The current (top) traces are 3 mV negative to the zero reference level. Calibrations, 40 mV; 2×10^{-6} A; 1·0 s for all records, except 2·0 s for (*a*) 25 and 50 mM-Mn^{2+}.

minutes to calcium-free ASW strongly suggested that calcium is required for the generation of overshooting events. Fig. 2 shows responses recorded intracellularly from a preparation bathed in calcium-free ASW containing 1 mM EGTA. The overshooting component of the responses elicited by Carb was abolished within 3 min (Fig. 2*b*). Subsequent responses, recorded up to 30 min later, exhibited a continuing reduction in amplitude. Overshooting responses were again elicited several minutes after the preparation was returned to control ASW (Fig. 2*e*).

Further experimental evidence that calcium is the main ion that contributes to the depolarizing responses was obtained by direct electrical stimulation of the myoepithelial cells. Only simple electrotonic responses were elicited when the preparation was bathed in calcium-free ASW. Fig. 1(*b*), from the same cell as Fig. 1(*a*), shows electrotonic responses recorded 6 min after exposure to calcium-free ASW. The effect was reversible upon return to control ASW (Fig. 1*c*). It is important to note that overshooting responses could be elicited by directly applied stimuli for several minutes after the solution was changed from calcium-containing to calcium-free ASW. The most plausible explanation for this result, and for the slow reduction in amplitudes of responses elicited by the application of Carb to the bath, is that sufficient calcium may remain in the preparation, probably within the highly branched t-tubules (Smith *et al.* 1973), to support responses to several stimuli.

The regenerative responses elicited by direct stimulation in calcium-containing ASW were blocked reversibly by 15–20 mM-Co^{2+} and essentially irreversibly by 10 mM-La^{3+}. (In 1 mM-La^{3+} the responses were prolonged but not completely abolished.) Finally, regenerative responses were elicited by direct stimulation in calcium-free ASW containing 1 mM-Ba^{2+} (Fig. 1*f*) and 10 mM-Sr^{2+} (Fig. 1*g*). All of

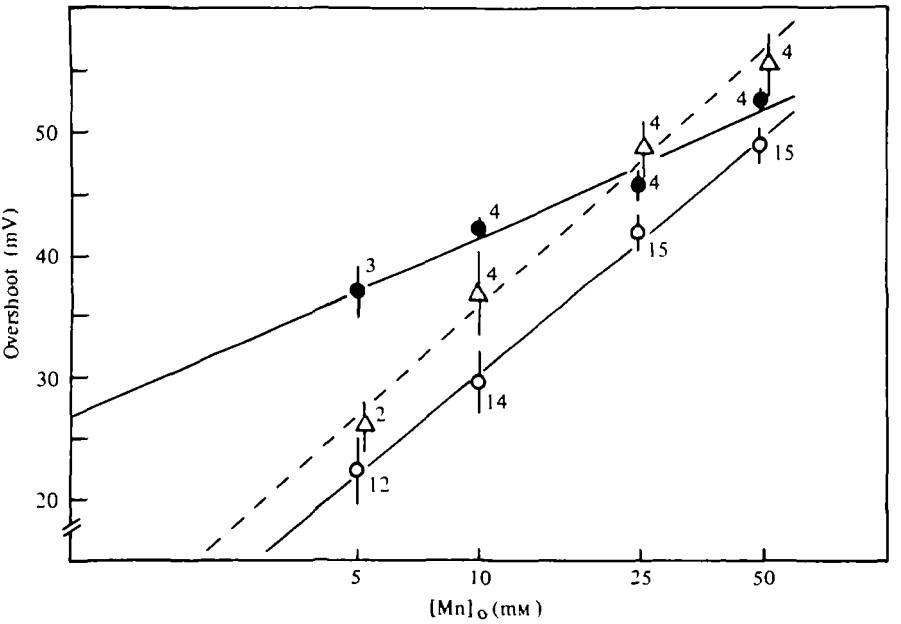

Fig. 4. Overshoots plotted against $\log_{10}$ $[Mn]_o$, pooled data. See test for details. Solid line, open symbols, Ca-free ASW (ten preparations); solid line, closed symbols, Ca-containing (10 mM) ASW (four preparations); interrupted line, Na- and Ca-free ASW (three preparations). Bars indicate standard errors of the mean; numbers indicate total number of cells tested.

these results support the conclusion that the overshooting events generated by the myoepithelial cells are calcium spikes.*

Replacement of Ca^{2+} by Mn^{2+}. In ASW in which calcium was replaced by an equimolar concentration (10 mM) of manganese, directly applied current pulses elicited responses of 77–117 mV amplitude and 0·42–16·4 s duration (25 preparations). The amplitudes of responses elicited in calcium-free ASW containing Mn^{2+} increased as the $[Mn]_o$. (Sample records are shown in Fig. 3*a*.) From pooled data, semilog$_{10}$ plots were made of the mean overshoots of responses against $[Mn]_o$; the slope for a tenfold change in $[Mn]_o$ in calcium-free ASW was 27·5 for solutions containing a constant concentration of divalent cations (ten preparations, open symbols and solid line of Fig. 4) and for solutions containing a constant $[Mg]_o$ (eight preparations). This value approaches that predicted by the Nernst equation for a membrane permeable to a divalent cation. Thus, these data indicate a dependence of the regenerative response on Mn^{2+} ions.

Permeation of calcium channels by Mn^{2+}. Responses were elicited by direct stimulation in solutions containing calcium and varying concentrations of Mn^{2+}. Sample

* Experimental difficulties were encountered when the external concentraction of calcium was varied in an attempt to show that the amplitude of the spike increased as the $[Ca]_o$. Spikes could not be elicited consistently at concentraticns of Ca^{2+} below the control level of 10 mM, and, at higher concentrations, the cells seemed to contract more vigorously than in control ASW; this often pushed the electrode out of the cell. In the two experiments in which a complete series of tests at three different concentrations of calcium (10–50 mM) was made on a single cell, plots of the amplitudes of the responses against the $\log_{10}$ $[Ca]_o$ resulted in slopes of 14·4 and 24·3 mV for a tenfold change in $[Ca]_o$.

records are shown in Fig. 3(*b*). Although the responses increased in amplitude as the $[Mn]_o$ in these solutions increased, the slope for a tenfold change in $[Mn]_o$ was less than that observed in calcium-free solutions. From pooled data, the slopes for a tenfold change in $[Mn]_o$ in ASW containing 10 mM-Ca^{2+} were 14·6 (constant divalent cation, four preparations; closed symbols and solid line of Fig. 4) and 15·1 (constant $[Mg]_o$, one preparation). The reduced slope in the presence of Ca^{2+} suggests a competition between Ca^{2+} and Mn^{2+} ions for the same channels.

In addition, responses were elicited in calcium- and sodium-free solutions containing varying external concentrations of Mn; the amplitudes of responses increased as the $[Mn]_o$ increased in a manner similar to that observed in calcium-free, sodium-containing solutions. Sample records are shown in Fig. 3(*c*). From pooled data, the change in overshoot amplitude for a tenfold change in $[Mn]_o$ in calcium- and sodium-free ASW was 29·6 (constant $[Mg]_o$, three preparations, interrupted line of Fig. 4). These data lend further support to the idea that the responses elicited in calcium-free manganese-containing solutions are the result of the opening of ion-specific channels.

Final evidence suggesting that Mn^{2+} enters the cells via calcium channels is that Co^{2+} ions at a concentration of 20 mM reversibly blocked the Mn^{2+} spike (in Ca-free ASW containing 10 mM-Mn^{2+}), and La^{3+} ions at a concentration of 10 mM blocked it irreversibly; these concentrations are in the same ranges as those found effective in blocking Ca^{2+} spikes.

Durations of directly elicited regenerative responses. The absolute durations of responses varied from preparation to preparation and within any single experiment. However, it was clear throughout this study that, in the presence of Mn^{2+} ions, the spikes elicited in both Ca-free and Ca-containing solutions were longer than those elicited in control ASW. The spikes elicited in Ca-free ASW containing Sr^{2+} or Ba^{2+} were also of longer duration than those elicited in control ASW. In addition, it was often observed that the durations of responses within a given test series increased as the $[Mn]_o$. This can be seen in the sets of responses illustrated in Fig. 3. (Note the different time calibration for Fig. 3(*a*), 25 and 50 mM-Mn^{2+}.) Further, at higher concentrations of Mn^{2+}, the durations at a given $[Mn]_o$ were consistently greater in Ca-free ASW than in Ca-containing ASW. This observation suggested that calcium may be required to activate repolarization and that Mn^{2+} competes with, but cannot replace, Ca^{2+} in this function. To test this idea, responses were elicited from two different preparations in solutions containing 10 mM-Mn^{2+} and increasing concentrations of Ca^{2+} (10–50 mM). Under these conditions the amplitudes of the responses did not increase with $[Ca]_o$; however, the durations of the responses consistently decreased as the $[Ca]_o$ increased. The records of Fig. 5, obtained from one preparation, show a reduction in duration from 2200 ms at 10 mM-Ca^{2+} to 350 ms at 50 mM-Ca^{2+}. This result is consistent with the idea that calcium ions are involved in the process of repolarization; as the $[Ca]_o$ increased, the prolonging effect exerted by Mn^{2+} was apparently diminished. In an attempt to further characterize the process of repolarization, tetraethylammonium (TEA) a substance known to block specifically potassium conductance in other animals, was applied to the proventriculus. Although TEA at a concentration of 25 mM seemed to prolong Ca spikes in some experiments, this effect was not found from preparation to preparation.

Contractile activity. The Mn^{2+} responses elicited in calcium-free ASW were no

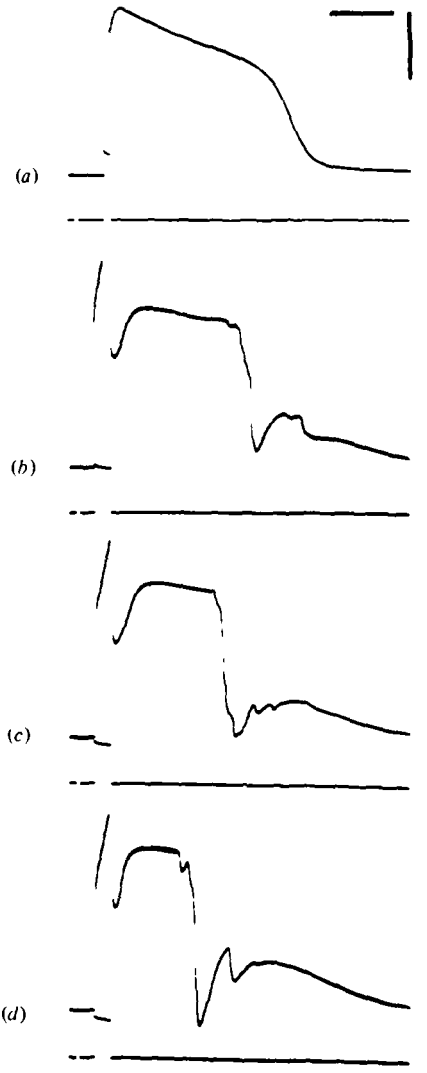

Fig. 5. Reduction in duration of directly elicited responses in solutions containing 10 mM-Mn^{2+} and increasing concentrations of Ca^{2+}: 10 (*a*), 20 (*b*), 30 (*c*), and 50 (*d*) mM. The portions of the records beyond the rapid falling phases may be contraction artifacts. Calibrations, 40 mV; 2×10^{-6} A; 1000 ms (*a*) and 400 ms (*b–d*).

associated with contractions. Correlations of electrical and mechanical activity of the myoepithelial cells are shown in Fig. 6. Because the cells are orientated radially around the lumen of the proventriculus (Smith *et al.* 1973) their contraction opens the lumen. In Fig. 6(*a*) the proventriculus and its electrical activity are shown when no stimulus was applied. In Fig. 6(*b*) the preparation was bathed in control ASW, a pulse was applied directly and both depolarization and contraction resulted (note the expanded central lumen). In Fig. 6(*c*) the preparation was bathed in calcium-free ASW containing 10 mM-Mn^{2+}; although the applied pulse elicited a large depolarization, it did elicit contraction and expansion of the lumen. These results suggest that the

initiation of contraction requires an influx of calcium ions from the extracellular space. Although Mn^{2+} can replace Ca^{2+} in generating spikes, Mn^{2+} apparently cannot replace Ca^{2+} in initiating contraction.

Other ions. All the divalent cations, tested, except Mn^{2+}, were found to block the directly elicited Ca spikes. Those ions that blocked the spike reversibly were Mg^{2+} (100 mM, one preparation), Co^{2+} (15–25 mM, three preparations) and Ni^{2+} (2·5–10 mM, four preparations). Zn^{2+} blocked the Ca-spike irreversibly (100 μM, two preparations).

DISCUSSION

The experiments presented in this paper show that the myoepithelial cells of the proventriculus of the marine polychaete worm *Syllis spongiphila* undergo regenerative depolarizations. Unlike several invertebrate muscle fibres which require the suppression of potassium conductance by the application of TEA to render them capable of undergoing all-or-none-action potentials (e.g. Fatt & Ginsborg, 1958; Washio, 1972; Fukuda & Kawa, 1977), the *Syllis* myoepithelial cells generate spikes in response to direct stimulation without treatment with TEA. On the basis of several criteria (Hagiwara, 1973) the overshooting depolarizations were shown to be calcium spikes. They occurred in low-Na solutions, were unaffected by TTX, were reversibly abolished in Ca-free solutions and were blocked in solutions containing Co^{2+} and La^{3+}; in addition, Ba^{2+} and Sr^{2+} supported responses in Ca-free ASW. The use of the insensitivity of the myoepithelial cells to TTX as support for the conclusion that the cells generate Ca-spikes may be criticized, since some Na-channels are known to be resistant to TTX (Harris & Thesleff, 1971; Hagiwara, 1973). However, since the amplitudes of directly elicited spikes were not obviously diminished when 95 % of the Na^+ in the bath was replaced by sucrose, it seems unlikely that Na^+ ions contribute greatly to the spikes generated by the myoepithelial cells.

The experiments also show that Mn^{2+} ions permeate the calcium channels. The overshoots of Mn^{2+} responses in Ca-free ASW increased as the $[Mn]_o$ (in both the presence and absence of Na^+), and this increase was diminished when calcium was present in the bathing medium. Further, the Mn spikes were blocked by Co^{2+} and La^{3+} at concentrations similar to those at which these ions blocked Ca spikes. All of these results suggest that both Ca^{2+} and Mn^{2+} ions permeate the membrane via the same sites. Of all the transition metals tested, only Mn^{2+} permeated the calcium channels. It is of interest that, of the divalent first transition series metals, Mn^{2+} exhibits the lowest heat of hydration (Basolo & Pearson, p. 86, 1967). It seems probable that only Mn^{2+} is sufficiently unstable to shed its waters of hydration and pass through the calcium channel. This hypothesis is supported by the fact that all of the Group IIA metals that pass through the calcium channel (Ca^{2+}, Sr^{2+} and Ba^{2+}) exhibit energies of hydration that are still lower than that of Mn^{2+} (Ochiai, 1977). (The ionic radius of Mn^{2+} is smaller than the radii of Sr^{2+}, Ba^{2+} and Ca^{2+} (Basolo and Pearson, p. 81, 1967)). Experimental evidence obtained in this study using two other transition metals, Ni^{2+} and Co^{2+}, also lends some support to the hypothesis. Ni^{2+} was effective at a lower concentration than Co^{2+} in reversibly blocking the Ca spike; Ni^{2+} exhibits a greater heat of hydration and is considered more stable among the transition metals than Co^{2+} (Ochiai, 1977). The results obtained using Zn^{2+} do not support the simp

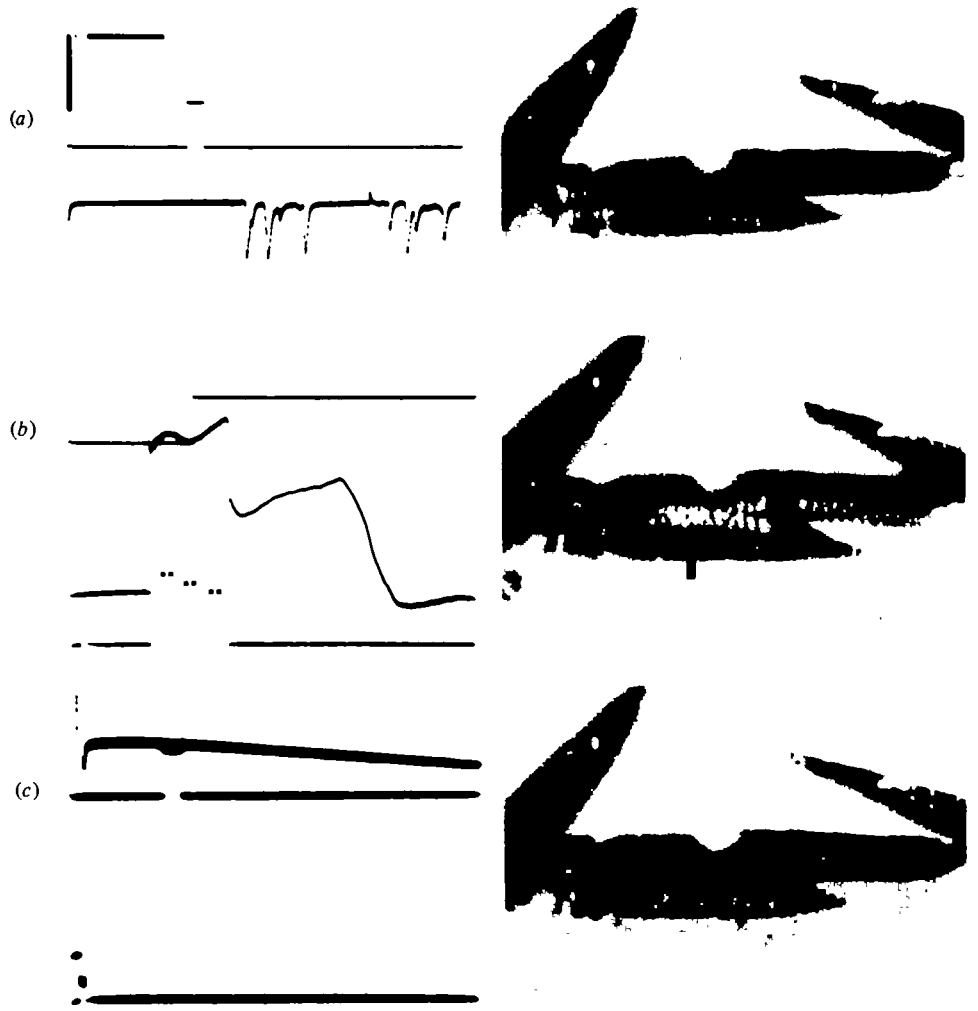

Fig. 6. Correlation of mechanical activity and intracellularly recorded electrical activity. Light micrographs (right) of the proventriculus, which is approximately 1·5 mm in length, were taken under various experimental conditions. Deflexions on the top traces at the left indicate the 0·5 s period during which the shutter of the camera was open. All records were taken from the same cell; responses in (*b*) and (*c*) were elicited by direct electrical stimulation. (*a*) The preparation was bathed in control ASW and no stimulus was applied; small hyperpolarizing junctional potentials occurred spontaneously; the central lumen of the proventriculus does not appear open. (*b*) The preparation was bathed in control ASW and a current pulse (bottom trace; peak of current pulse indicated by dotted lines) elicited a depolarizing response which was associated with opening of the lumen (see arrow). (*c*) The preparation was bathed in Ca-free ASW containing 10 mM-Mn and the applied current pulse elicited a large depolarization of long duration (repolarization is not shown); this response was not associated with opening of the lumen. Calibrations: 20 mV (*a*) and 40 mV (*b*) and (*c*); 1×10^{-8} A; 2 s (*a*, *c*) and 100 ms (*b*).

form of the hypothesis; Zn^{2+}, whose energy of hydration is similar to that of Co^{2+} (Basolo & Pearson, p. 86, 1967), blocked the Ca spike irreversibly at a concentration of 100 μM. Studies now in progress, using these and other metals, may help to reveal some of the nature of the calcium channel. Since Mn^{2+} appears to replace Ca^{2+} in the generation of spikes in certain cardiac cells (Ochi, 1975), the *Syllis* myoepithelial cell membrane, and some other invertebrate preparations (Fukuda & Kawa, 1977; Hagiwara & Miyazaki, 1977) may provide models for studies of certain mammalian cardiac membrane phenomena.

The results presented indicate that Ca^{2+} ions are involved not only in the generation of the overshooting depolarization, but also in the initiation of both contraction and repolarization. Although Mn^{2+} can replace Ca^{2+} in generating spikes, it apparently cannot replace Ca^{2+} in initiating contraction and repolarization; indeed, it appears that Mn^{2+} competes with Ca^{2+} in initiating repolarization.

The durations of responses elicited in Ca-free ASW containing Mn^{2+}, Sr^{2+} and Ba^{2+} all exceeded those elicited in control ASW. Experiments in which TEA was used in an attempt to demonstrate the occurrence of an increased permeability to potassium during the spikes did not clearly reveal any effect exerted by this compound. It is possible that the potassium conductance in the myoepithelial cells is not sensitive to TEA; some neurones exhibit several potassium conductances, with different levels of sensitivity to TEA (Barrett & Barrett, 1976; Thompson, 1977). The effect of Ba^{2+} in prolonging action potentials has been observed in several preparations; Sperelakis, Schneider & Harris (1967) suggested that barium acts by specifically reducing the potassium conductance. Since repolarization is delayed in the *Syllis* myoepithelial cells in Ca-free ASW containing Sr^{2+}, Ba^{2+} or Mn^{2+}, it is attractive to suggest that repolarization results at least in part from a calcium-activated potassium current (Meech, 1974; Meech & Standen, 1975; Thompson, 1977). A TEA-insensitive, Ca-activated potassium current has been reported. Thompson (1977), in voltage-clamp studies on nerve cell bodies of *Tritonia diomedia*, demonstrated three different potassium currents; one of these, the C current, is Ca-dependent, is apparently unaffected by TEA and is reversibly blocked by Co^{2+} and Mn^{2+} ions. In the *Syllis* myoepithelial cells, Mn^{2+} (Sr^{2+} and Ba^{2+}) would compete with Ca^{2+} in its role in potassium activation, and would thereby prolong the depolarizations generated by these cells.

I am grateful to Mr F. McKenzie and Mr G. Garcia for providing me with animals. I wish to thank Dr R. F. Olivo for critically reading the manuscript and Drs S. W. Kirtley and C. Levin for helpful discussions. This work was supported by National Institutes of Health Grant 1 RO1 NS12196.

REFERENCES

ANDERSON, M. (1977). Mn^{++} can replace Ca^{++} in generating spikes in myoepithelial cells. *Am. Zool.* **17**, 962.

ANDERSON, M. & DEL CASTILLO, J. (1976). Electrical activity of the proventriculus of the polychaete worm *Syllis spongiphila*. *J. exp. Biol.* **64**, 691–710.

ANDERSON, M. & MROSE, H. (1978). Chemical excitation of the proventriculus of the polychaete worm *Syllis spongiphila*. *J. exp. Biol.* **75**, 113–122.

BARRETT, E. F. & BARRETT, J. N. (1976). Separation of two voltage-sensitive potassium currents, and demonstration of a tetrodotoxin-resistant calcium current in frog motoneurones. *J. Physiol* **255**, 737–774.

BASOLO, F. & PEARSON, R. G. (1967). *Mechanisms of Inorganic Reactions*, 2nd ed., pp. 81 and 86. New York: John Wiley.

DEL CASTILLO, J., ANDERSON, M. & SMITH, D. S. (1972). Proventriculus of a marine annelid: muscle preparation with the longest recorded sarcomere. *Proc. natn. Acad. Sci. U.S.A.* **69**, 1669–1672.

FATT, P. & GINSBORG, B. L. (1958). The ionic requirements for the production of action potentials in crustacean muscle fibres. *J. Physiol.* **142**, 516–543.

FUKUDA, J. & KAWA, K. (1977). Permeation of manganese, cadmium, zinc, and beryllium through calcium channels of an insect muscle membrane. *Science, N.Y.* **196**, 309–311.

GILLESPIE, J. S. & MCKNIGHT, A. T. (1976). Adverse effects of tris hydrochloride, a commonly used buffer in physiological media. *J. Physiol.* **259**, 561–573.

HAGIWARA, S. (1973). Ca spike. *Adv. Biophys.* **4**, 71–102.

HAGIWARA, S. & MIYAZAKI, S. (1977). Ca and Na spikes in egg cell membrane. In *Cellular Neurobiology* (ed. Z. Hall, R. Kelly and C. F. Fox). pp. 147–158. New York: Alan R. Liss.

HAGIWARA, S. & NAKA, K.-I. (1964). The initiation of spike potential in barnacle muscle fibres under low intracellular Ca^{++}. *J. gen Physiol.* **48**, 141–162.

HAGIWARA, S. & NAKAJIMA, S. (1966). Differences in Na and Ca spikes as examined by application of tetrodotoxin, procaine and manganese ions. *J. gen. Physiol.* **49**, 793–806.

HAGIWARA, S. & TAKAHASHI, K. (1967). Surface density of calcium ions and calcium spikes in the barnacle muscle fiber membrane. *J. gen. Physiol.* **50**, 583–601.

HARRIS, V. B. & THESLEFF, S. (1971). Studies on tetrodotoxin resistant action potentials in denervated skeletal muscle. *Acta physiol. Scand.* **83**, 382–388.

HUTTER, O. F. (1952). Effect of choline on neuromuscular transmission in the cat. *J. Physiol.* **117**, 241–250.

KERKUT, G. A. & GARDNER, D. R. (1967). The role of calcium ions in the action potentials of *Helix aspersa* neurones. *Comp. Biochem. Physiol.* **20**, 147–162.

MAENO, T., EDWARDS, C. & ANRAKU, M. (1977). Permeability of the endplate membrane activated by acetylcholine to some organic cations. *J. Neurobiol.* **8**, 173–184.

MEECH, R. W. (1974). Calcium influx induces a post-tetanic hyperpolarization in *Aplysia* neurones. *Comp. Biochem. Physiol.* **48** A, 387–395.

MEECH, R. W. & STANDEN, N. B. (1975). Potassium activation in *Helix aspersa* neurones under voltage clamp: a component mediated by calcium influx. *J. Physiol.* **249**, 211–239.

OCHI, R. (1975). Manganese action potentials in mammalian cardiac muscle. *Experimentia* **31**, 1048–1049.

OCHIAI, E.-I. (1977). *Bioinorganic Chemistry. An Introduction*, p. 55. Boston: Allyn and Bacon.

REUTER, H. (1973). Divalent cations as charge carriers in excitable membranes. *Progr. Biophys. Molec. Biol.* **26**, 1–43.

SMITH, D. S., DEL CASTILLO, J. & ANDERSON, M. (1973). Fine structure and innervation of an annelid muscle with the longest recorded sarcomere. *Tissue & Cell* **5**, 281–302.

SPERELAKIS, N., SCHNEIDER, M. F. & HARRIS, E. J. (1967). Decreased K^+ conductance produced by Ba^{++} in frog sartorius fibers. *J. gen. Physiol.* **50**, 1565–1583.

THOMPSON, S. H. (1977). Three pharmacologically distinct potassium channels in molluscan neurones. *J. Physiol.* **265**, 465–488.

WASHIO, H. (1972). The ionic requirements for initiation of action potentials in insect muscle fibers. *J. gen Physiol.* **59**, 121–134.

WELSH, J. H., SMITH, R. I. & KAMMER, A. E. (1968). *Laboratory Exercises in Invertebrate Physiology*, 3rd ed., p. 192. Minneapolis: Burgess.

WILSON, W. A., CLARK, M. T. & PELLMAR, T. C. (1977). Tris buffer attenuates acetylcholine responses in *Aplysia* neurones. *Science, N.Y.* **196**, 440–441.