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ELECTRICAL CURRENTS ASSOCIATED WITH RHYTHMIC CONTRACTIONS OF THE BLASTODERM OF THE MEDAKA, *ORYZIAS LATIPES*

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Abstract—1. We used a vibrating probe to measure extracellular electrical currents near the surface of dechorionated *Oryzias latipes* eggs as contraction waves moved slowly across the blastoderm.

2. Although we found no detectable current outside dechorionated embryos, we recorded large current pulses near the edge of wounds made in the surface of the blastoderm.

3. The maximum net inward current—or in some cases, the least net outward current—correlated temporally with the contraction of cells near the edge of the wound.

4. The current pulses were superimposed on steady currents of variable magnitude and polarity.

5. We discuss possible mechanisms for the initiation and propagation of the contraction wave.

INTRODUCTION

The blastoderm of the *Oryzias latipes* embryo begins to contract rhythmically during epiboly and continues to do so until late in development (Yamamoto, 1975; Robertson, 1979; Fluck *et al.*, 1983). When they first begin, the contractions arise in the extra-embryonic portion of the blastoderm that is centered at 180° longitude from the embryonic shield (Barber *et al.*, 1987), in what Kageyama (1982) called the ventral region of the blastoderm. Barber *et al.* (1987) called the cells in this region "pacemaker" cells. When a portion of the blastoderm (470 × 200 μm) containing about 130 enveloping layer (EVL) cells is removed from this region, the rhythmic contractions stop. Comparable wounds in other parts of the blastoderm have no apparent effect on the contractions (Barber *et al.*, 1987). Contraction waves leave the pacemaker region once every 60–120 sec and propagate along the blastoderm at a velocity of 14–54 μm/sec (Fluck *et al.*, 1984; Barber *et al.*, 1987). Although Fluck *et al.* (1983) concluded that the rhythmic contractions reside within EVL cells, Fluck (unpublished data) has recently found another layer of cells—the stellate layer—closely apposed to the basal surface of the EVL (Armstrong, 1980). His observation raises the possibility that both the stellate layer and the EVL may contract rhythmically.

In the present study, we used the extracellular vibrating probe (Jaffe and Nuccitelli, 1974) to measure extracellular electrical currents near the surface of the *Oryzias latipes* embryo during rhythmic contractions. Although we detected no steady or pulsing currents outside the intact embryo, we found large, pulsing currents near wounds we made in the surface of the blastoderm.

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MATERIALS AND METHODS

Organisms

The growth of adults and embryos of *Oryzias latipes* and the proteolytic dechorionation of embryos have been described previously (Fluck, 1978; Fluck *et al.*, 1983, 1984; Barber *et al.*, 1987). After dechorionation, we grew the embryos in embryo rearing medium (17 mM NaCl, 0.4 mM KCl, 0.3 mM $CaCl_2$, 0.67 mM $MgSO_4$) until they reached developmental stage 14 or 15 (approximately one-third to one-half epiboly; Yamamoto, 1975).

Current measurements

We measured extracellular currents with a vibrating probe, which can reliably and accurately determine both the amplitude and direction of a current (Jaffe and Nuccitelli, 1974), using probes that had platinum black balls with diameters of 25–30 μm and a similar range of vibration amplitudes. All measurements were made at 20–23°C. We recorded the contractile activity of the embryos with a Gyyr timelapse video cassette recorder equipped with a time–date generator.

To measure the current near the surface of dechorionated embryos, we placed embryos in a Petri dish of embryo rearing medium. However, in this medium the embryos rolled from side to side—towards and away from the vibrating electrode—during rhythmic contractions, making it difficult to obtain reliable electrical measurments. We reduced the extent of this rolling by adding methyl cellulose (2%, 400 centipoises; Sigma) to the medium. In 2% methyl cellulose, the amplitude and speed of the rolling were decreased enough that we could, when necessary, translate the electrode to keep it at a fixed distance from the surface of the embryo.

To measure the current near wounded embryos, we placed dechorionated embryos in 2% methyl cellulose in isolating medium (143.5 mM NaCl; 5.4 mM KCl; 0.8 mM $MgSO_4$; 1.8 mM $CaCl_2$; 10.0 mM HEPES, pH 7.4; Fluck *et al.*, 1984) and wounded them by removing a portion of the

EVL andf stellate layer with watchmaker's forceps. To estimate the number of cells we had removed from the EVL, we measured the size of the wound (from the videotape record) and used Kageyama's (1982) values for the average apical surface area of EVL cells at stages 14 and 15, 730 and 1450 μm^2, respectively. This estimate was difficult to make because: (1) our perspective usually permitted us to see the wound only in profile and (2) the wounds began to close soon after they were made, typically closing by 30% within a few minutes (Barber *et al.*, 1987). We wounded some embryos more than once, either enlarging the first wound or making a second wound in a different area. We typically began recording electrical signals within 5 min after wounding and recorded for an average of 12 min (range = 6–33 min). We positioned the probe at the edge of a wound so that it vibrated along a normal to the surface of the egg (Fig. 1a).

RESULTS

Intact embryos

We found no detectable (i.e. $<0.1\ \mu A/cm^2$) steady or pulsing currents anywhere near the surface of the embryos.

Wounded embryos

Wounding had very little effect on the appearance or timing of the rhythmic contractions. In only one case (no. 5b) did the cells near the edge of the wound stop contracting; moreover, the periods we measured [Table 1; 92 ± 17 sec ($\bar{x} \pm$ SD, $n = 13$)] were similar to those previously reported by Fluck *et al.* (1983).

We measured electrical currents near 29 wounds in

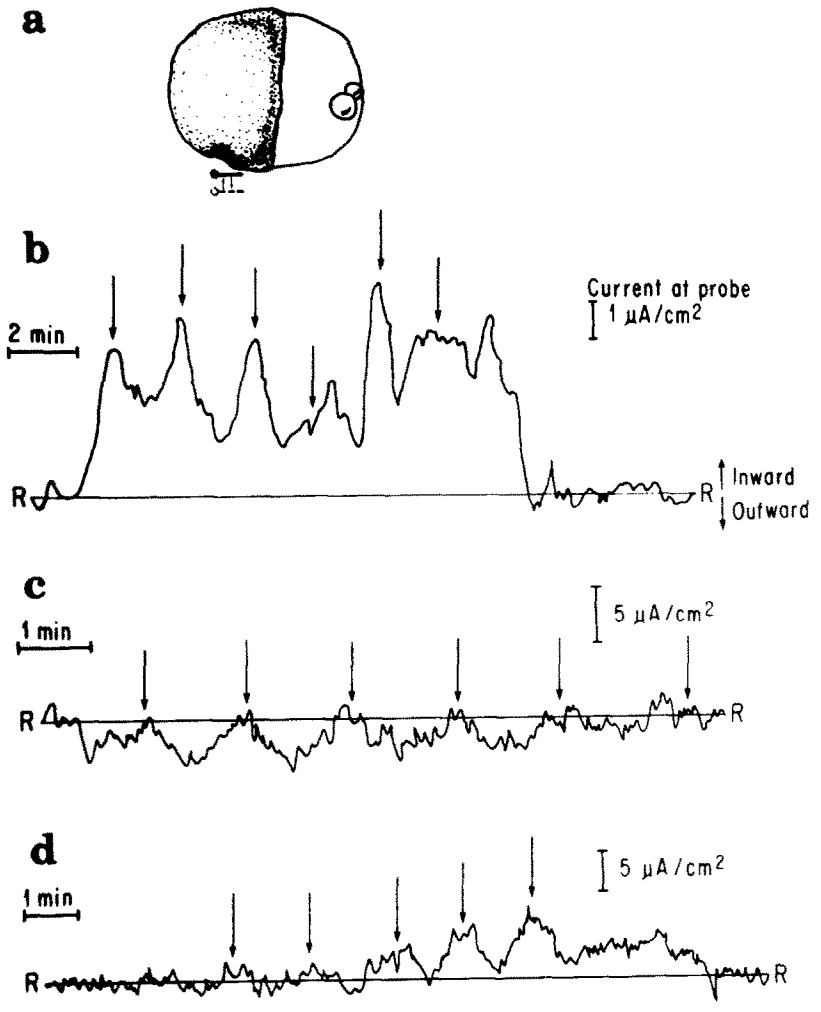

Fig. 1. Currents recorded near the edge of wounds in the surface of the blastoderm. (a) We positioned the probe at the edge of a wound so that it vibrated along a normal to the surface of the egg. In this embryo, the blastoderm has expanded to cover one half of the egg (stage 15). The diameter of the egg is approximately 1 mm. (b–d) Current records from three embryos. After placing the probe at a reference position distant from the egg (R on the current records), we positioned the probe near the edge of a wound for several minutes and then returned to the reference position. Embryo nos 7 and 26, (b) and (c), respectively, were wounded in the pacemaker region, while no. 23 (d) was wounded lateral to the pacemaker region. In (b) and (d) the net current was always inward; in (c) the polarity of the net current changed direction during the contractions. Arrows mark the time of contraction of cells near the edge of the wound.

Table 1. Extracellular currents near wounds in the blastoderm of *Oryzias latipes* embryos

No.	No. of EVL cells removed	Pulsing current?	Period of pulses (sec)	Magnitude of current (μA/cm^2) Early range	Peak-to-trough	Strength of contraction*
Wounded in pacemaker region						
6	14	Yes	110	0–10 in	$\leq$7	++
7	9	Yes	120	1–5 in	$\leq$4	++
9b	20	Yes	110	10–15 out	$\leq$4	++
10b	31	Yes	80	2–3 out	$\leq$1	+
11	14	Yes	120	3 in–6 out	$\leq$5	++
19a	17	Yes	82	0–21 in	$\leq$12	++
26	186	Yes	90	1 in–3 out	$\leq$4	++
27	28	Yes	80	1 in–3 out	$\leq$4	++
33	38	Yes	84	1–55 out	$\leq$48	++
Wounded near pacemaker region						
5b	11	No	—	$\leq$0.4†	—	0
8a	10	No	—	$\leq$0.4	—	++
9a	20	No	—	0 1/2 out	—	+
10a	80	No	—	1–2 out	—	+
21	123	Yes	90	1 in–4 out	$\leq$5	++
23	107	Yes	84	0–8 in	$\leq$8	++
24	55	Yes	80	2–5 out	$\leq$3	++
25	44	Yes	70	1–4 in	$\leq$3	++

*Strong rhythmic contractions, ++; weak rhythmic contractions, +; no rhythmic contractions, 0. Italicized case numbers are illustrated in Fig. 1.
†Cells near the edge of the wound did not contract rhythmically.

24 embryos. Of these, we rejected 12 cases because: (1) the probe was not positioned correctly, (2) the embryo rolled away from the probe or rolled back and forth, or (3) the wound was not clearly visible on the video monitor.

We detected pulsing currents near all nine wounds we made in the pacemaker region (Table 1; Fig. 1b, c). In eight cases, we recorded both high amplitude mechanical and electrical pulses; and in one (no. 10b), we recorded both low amplitude mechanical and electrical pulses. The peak-to-trough amplitude of the pulses ranged from 1 to 48 μA/cm^2 (10 ± 15 μA/cm^2, $\bar{x} \pm$ SD; $n = 9$).

The current pulses were superimposed upon a steady current that varied in polarity and magnitude. During pulsing the current ranged from 0–10 μA/cm^2 into the embryo, to 3 μA/cm^2 in to 6 μA/cm^2 out, to 10–15 μA/cm^2 out. The maximum net inward current—or in some cases the least net outward current—always occurred just as the cells near the edge of the wound were contracting (Fig. 1).

In four of the eight wounds we made lateral to the pacemaker region, we recorded both high amplitude mechanical and electrical pulses (5 ± 2 μA/cm^2, $\bar{x} \pm$ SD; range = 3–8 μA/cm^2; $n = 4$). In another (no. 5b), we saw no electrical pulses or rhythmic contractile activity in cells near the edge of the wound; and in two others (no. 9a, 10a), we saw very weak contractions and no electrical pulses.

DISCUSSION

We observed two types of electrical currents near the edges of wounds: pulsing currents and steady currents. The maximum net inward current (or in some cases the least net outward current) usually (13/17) occurred during maximum contraction of cells near the edge of the wound; and in the remaining four cases, both rhythmic contractile activity and the electrical pulses were absent or weak.

We have considered three mechanisms for how blastoderm cells might initiate and propagate the contractions. First, like pacemaker cells in cardiac muscle and other tissues (Carpenter, 1982), pacemaker cells in the blastoderm may depolarize spontaneously and generate an action potential that is conducted to other cells. This possibility is consistent with the current pulses we report here and with the observation that rhythmic contractions stop when solutions containing 100 mM K^+ are microinjected into the segmentation cavity, which is in contact with the basolateral surface of the EVL (Fluck *et al.*, 1984). Furthermore, the rhythmic contractions of detached blastoderms are also very much reduced in medium containing $[K^+] \geq 25$ mM (Sguigna *et al.*, 1987). However, it is unlikely that the contractions are propagated by means of an action potential that moves out of the pacemaker region and into adjacent cells, because the conduction velocity of the contraction waves is so low. In epithelia that conduct action potentials, the conduction velocities typically range from 15 to 35 cm/sec (Mackie and Passano, 1968), or about four orders of magnitude faster than the blastoderm propagates contractions.

Alternatively, the contraction wave may be propagated by a wave of increased cytosolic free Ca^{2+}. Such waves traverse fertilized eggs of *Oryzias latipes* (Gilkey *et al.*, 1978) and *Xenopus laevis* (Busa and Nuccitelli, 1985), with velocities of $\sim$12 and $\sim$10 μm/sec, respectively. In *Xenopus*, the wave of increased free Ca^{2+} may be responsible for the external activation current, which also propagates across the zygote at a similar velocity (Kline and Nuccitelli, 1985) and which is similar in magnitude to the currents reported in the present study. In the *Oryzias* blastoderm, such a wave of increased Ca^{2+} could be propagated from cell to cell via gap junctions (Lentz and Trinkaus, 1971; Bennett and Trinkaus, 1970).

Calcium ions are required for rhythmic contraction of the medaka blastoderm (Sguigna *et al.*, 1987). In detached blastoderms, the optimal $[Ca^{2+}]$ for rhythmic contractions is $\sim$1 mM; the contractions stop (or their amplitude is very much reduced) in medium containing 0.18 or 18 mM Ca^{2+} or in medium con-

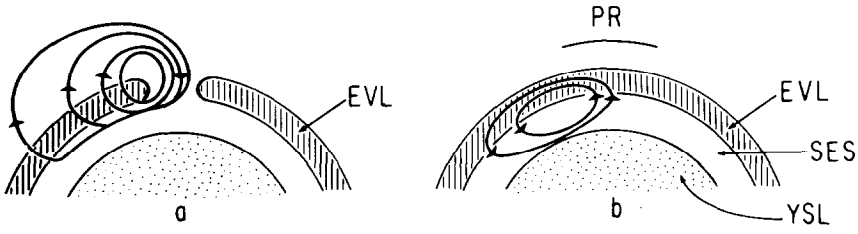

Fig. 2. Models of pulsatile component of current during peak of contraction. (a) Wounded (from data). (b) Unwounded (speculative). In both cases, current (including Ca^{2+} ions?) would leak across the basolateral membrane of EVL cells during contraction. However, in a wounded egg, at least some of this would be balanced by current pumped out of remote apical membranes; while in unwounded ones, it would be entirely balanced by current pumped out of remote basolateral membranes. The models would apply both to pacemaker cells (shown here) and to cells outside the pacemaker region. EVL, enveloping layer; PR, pacemaker; SES, subepithelial space; YSL, yolk syncytial layer.

taining 1 mM La^{3+}. Intracellular Ca^{2+} is also important for the contractions, because they are inhibited by TMB-8 and caffeine (Sguigna *et al.*, 1987), both of which modify the release of Ca^{2+} from intracellular compartments. In preliminary experiments, we found that 1 mM La^{3+} abolishes both electrical pulsing and the contraction of cells near the wound edge. If a wave of free cytosolic Ca^{2+} accompanies the contraction waves, it may be possible to use aequorin (Gilkey *et al.*, 1978) or FURA-2 (Tsien *et al.*, 1985) to visualize it.

A third possible mechanism of propagation of the contraction wave is stretch: cells outside the pacemaker region may be caused to contract as a result of being stretched when nearby cells contract. Stretch triggers contraction in many types of muscle (Bozler, 1947; Bulbring, 1955; Prosser *et al.*, 1959); and Odell *et al.* (1981; see also Oster and Odell, 1984) have proposed a model for epithelial morphogenesis in which contraction waves are propagated by stretch. Of course, such a wave could be accompanied by changes in membrane potential and/or by a wave of increased cytosolic Ca^{2+}.

The current pulses were often superimposed on a steady current. This current was sometimes similar in magnitude and polarity to the large steady current that enters wounds made in the EVL of *Fundulus* embryos (Jaffe *et al.*, 1986), but in the present study the steady current often left, rather than entered, the wound. Further study is necessary to characterize the steady current and to determine the basis for the variability in the size and direction of this current.

We found no detectable currents near the surface of embryos that had not been wounded. This presumably reflects the extremely high impermeability and high electrical resistance of the apical surface of the enveloping layer (Prescott, 1955; Bennett and Trinkaus, 1970; Dunham *et al.*, 1970). Presumably, the large current densities measured near the small wound area were balanced by smaller ones spread out over the large area of enveloping layer far from the wound (Fig. 2a). In the absence of a wound, one could imagine comparable currents confined to the subepithelial space (Fig. 2b).

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