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TRANSMEMBRANE ELECTRICAL ACTIVITY OF THE SINGLE CELL LAYERED HEART OF "SEA POTATO"

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The heart of *Boltenia ovifera* (sea potato), like that of most tunicates, consists of a tubular structure which propels blood through the circulatory system by means of peristaltic contractions. The electronmicrograph in Figure 1 shows that the tunicate heart is composed of a single layer of myocardial cells interconnected by specialized junctions which appear also to isolate the inner from the outer surface membranes of the cells. Myofilaments and abundant mitochondria are located adjacent to the "endocardial" or luminal surface of the cells, consistent with our observation (MDIBL Bull., 1973 and Science, 1974) that only the luminal membrane of this heart appears to play an active role in excitation-contraction coupling processes. Large nuclei are located near the extraluminal surface. The luminal membrane is covered by a layer of amorphous ground substance whereas the extraluminal membrane is devoid of any such covering.

In previous reports (MDIBL Bull., 1972 and 1973, and Science, 1974), we studied some of the contractile and electrical properties of the tunicate heart using a perfused preparation in which a portion of the tubular heart was suspended in a bath between two cannulae. In these experiments the isolated heart was cut along the raphe to form a sheet and secured with a silicone grease coated clamp over an aperture connecting the two compartments of a modified Ussing chamber. Luminal and extraluminal surfaces of the

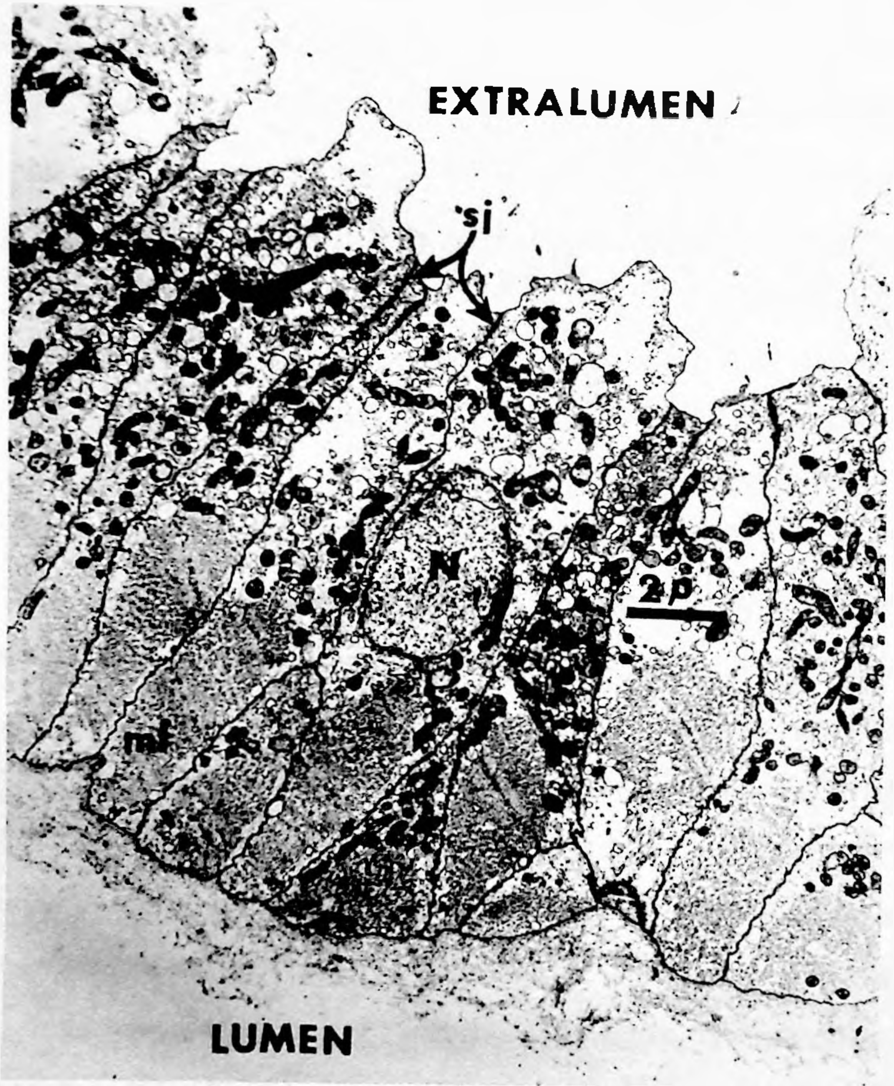

Figure 1: Electronmicrograph of a section of the "sea potato" heart, demonstrating the single cell layer nature of the myocardium. Myofibrils (mf) are located near the luminal surface, abundant mitochondria (m) primarily in the central portion of the cell, and prominent nuclei (N) towards the extraluminal surface. Specialized junctions (sj) interconnect adjacent cells and appear to insulate the luminal from the extraluminal aspects of the myocardium.

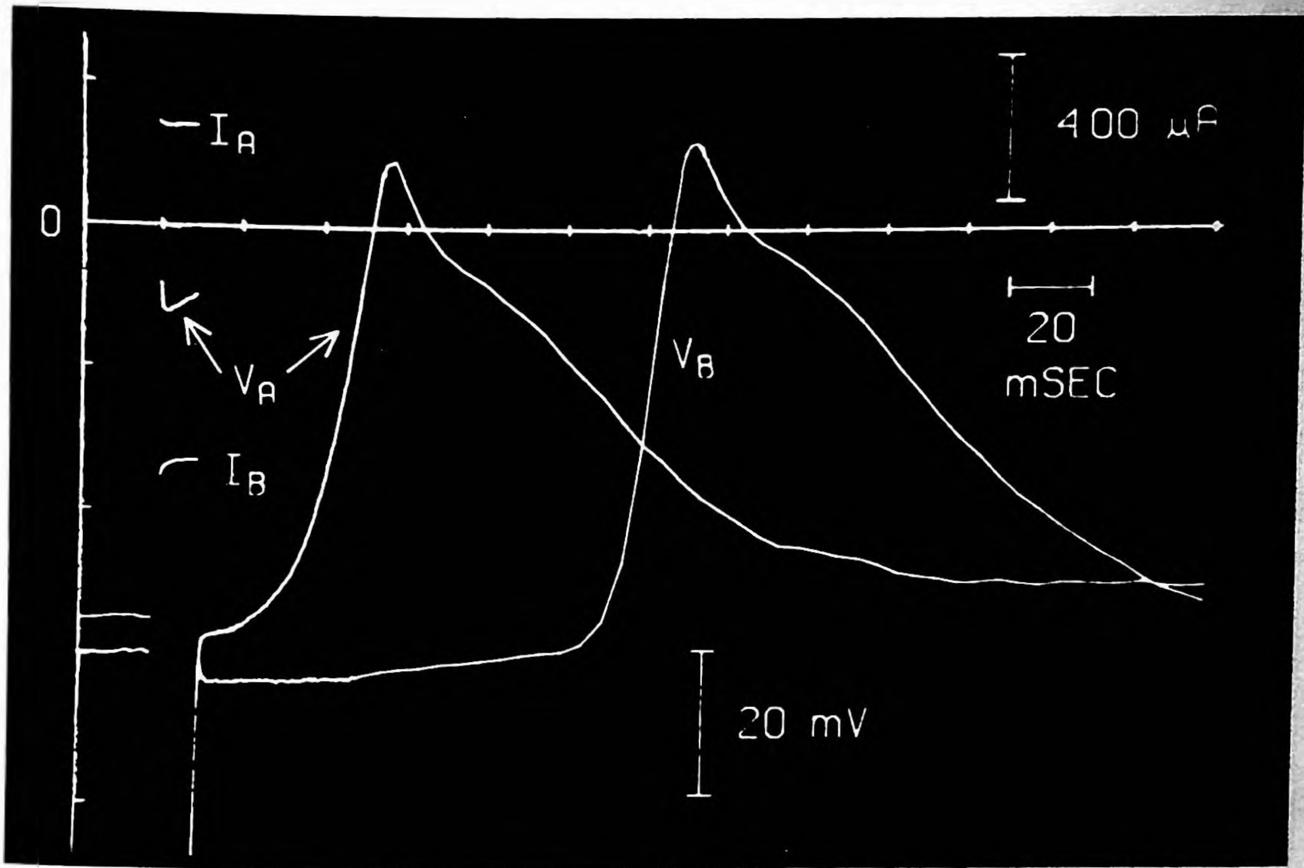

Figure 2: Action potentials following short (5 msec) current pulses depolarizing and hyperpolarizing the luminal membrane. The action potential V_B following the hyperpolarizing current pulse I_B does not occur until after a considerable delay, during which the cell gradually depolarizes before "breaking" into an action potential. This behavior is characteristic of anodal break excitation. The action potential V_A following a depolarizing current pulse I_A occurs immediately after the stimulus.

heart could be perfused independently. The standard perfusate consisted of a solution containing 365 mM NaCl, 10 mM KCl, 10 mM $CaCl_2$, 10 mM $MgCl_2$. It was found that if the solutions were cooled to 15°C (previous experiments were at room temperature), the survival time of the preparation was increased markedly. Two Ag/AgCl electrodes for current passing and voltage sensing respectively were placed in each compartment adjacent to either surface of the heart. Glass microelectrodes filled with 3M KCl were used to record intracellular potential.

Measurement of transcellular resistance by passing small current pulses through the sheet of myocardial tissue and measuring the resulting voltage drop across an independent set of electrodes disclosed a relatively low resistivity (∿100 Ω-cm^2). Consistent with this observation no appreciable transcellular action potential could be recorded between the voltage sensing electrodes following a superthreshold stimulus. Because of the fragile nature of the heart it was not clear whether the low resistivity actually represented anatomic low resistance extracellular pathways between adjacent myocardial cells or resulted from a "shunt" at an area of the preparation which had been inadvertently injured. Preliminary attempts to map the distribution of potential over the surface of the preparation however failed to identify any areas of localized high current density, indicative of a discrete "shunt."

Full size action potentials (70-80 mV in amplitude and 150-200 msec in duration) were recorded using KCl-filled microelectrodes (Figure 2). The action potentials could be suppressed by application of 10^{-6}M TTX. Action potentials following current pulses hyperpolarizing the luminal membrane occurred at the break of the current pulse suggesting anodal break excitation and directly demonstrating that only the luminal membrane is electrically excitable. The anodal break action potentials were graded with respect to stimulus magnitude and duration consistent with: 1) inactivation of a regenerative sodium system on varying the degree of hyperpolarization; and, 2) electrotonic contribution to the upstroke of the action potential by the depolarized extraluminal membrane. Action potentials resulting from current pulses depolarizing the luminal membrane were "all-or-none" in character. In several fresh preparations the contractile response (monitored visually) also appeared to be "all-or-none." With time however mechanical activity became graded on application of increasing current pulses. The development of graded contractions, associated with the appearance of graded electrotonic depolarizations in many cells of the preparation, appeared to represent electrotonically-conducted action potentials from other regions of the myocardium. It was felt that the graded contractile response is not an intrinsic property of the myocardium but results from a failure of an action potential to be uniformly propagated throughout the myocardium under certain adverse conditions such as tissue injury.

The tunicate heart may be characterized as a single cell layer myocardium with one "excitable" membrane. A regenerative TTX sensitive transport system (probably sodium) seems to be present only at the luminal membrane. The contractile elements are located on the luminal surface and show striations similar to those seen in the skeletal muscle. A low transcellular resistivity seems to be a feature of the myocardium and not related to tissue injury. Electrical activity appears to be "all-or-none" and the graded contractile response probably represents an artifact of aberrant conduction patterns in the presence of factors such as tissue injury.

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