CULTURED EMBRYONIC MOUSE HEART CELLS AS MODELS FOR *T. CRUZI*-MUSCLE CELL INTERACTION: CHARACTERIZATION OF SPONTANEOUS FIRING AND CHRONOTROPIC RESPONSES TO NOREPINEPHRINE

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Primary monolayer cultures of heart muscle cells (HMC) were characterized with respect to their spontaneous-firing properties and the chronotropic response to norepinephrine (NE). All the experiments were made with culture cells placed in a culture dish with Eagle's medium plus serum and 2,5 mM $CaCl_2$ adjusted to pH 7,2 and observed at an inverted microscope (PRION) equiped with phase-contrast optics and the temperature maintained at 37°C. Trans-membrane voltage was monitored through conventional 2,5 M - KCL - filled glass micropipettes with tip resistances of 40 to 60 M, coupled to a hydraulic and mechanical micromanipulators to permit good impalement.

Microelectrodes were allowed to seal-in for 5 minutes prior to data collection. Measurements on the action potential included frequency, overshoot (OS), resting potential (RP) or maximum distolic potential (MDP) and duration at 50% repolarization (APD50).

Cultures (2 to 5-day-old) maintained in Dulbecco's medium + horse serum and 2.5 mM $CaCl_2$, pH 7.2 at 37°C presented synchronism within a cluster and age-independent variability in action potential morphology. Parameters describing the action potential were analyzed in cells of different ages and no significant differences between 2 and 5-day-old cultures were detected. The overshoot varied from 8.5 ± 1.7 mV to 12.6 ± 3.7 mV; the maximum diastolic potential from -46 ± 19 mV -57.6 ± 16.8 mV; action potential duration measured at 50% amplitude ranged from 94.4 ± 66.4 ms to 114.8 ± 33 ms, and the spontaneous firing rate varied from 116 ± 11 to 181 ± 7bpm. In some experiments the cultures were maintained in a saline solution (mM: 137 NaCl, 4.7 KCl, 1.0 $MgCl_2$, 13 $NaHCO_3$, 1.0 NaH_2PO_4, 2.5 $CaCl_2$, 5 glicose), gassed with 5% CO_2/95% O_2, pH 7.2. This change in suffusion medium did not alter the results described above. Preliminary results obtained in *T. cruzi*-infected cultures showed a high incidence of assynchrony and arrythmias. A decrease in maximum diastolic potential could also be observed, without a significant change in mean-frequency.

In a separate group of experiments, coverslips (n=40) were placed in a laminar flow chamber mounted on the microscope stage and suffused with the saline solution described above. Spontaneously beating single cells or clusters were then visually monitored before and during exposure to pulses of NE (10-10M), and the frequency of spontaneous contractions increased as the NE concentration was increased. The cultures also showed the phenomenon of desensitization, since the responses decreased with sucessive NE applications. Preliminary results obtained with *T. cruzi*-infected cultures indicate a decreased sensitivity to NE. We conclude that cultures of heart muscle cells obtained from embryonic mice constitute a suitable model for a systematic study of both electrophysiological and pharmacological phenomena.

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