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Paced monophasic and biphasic waveforms alter transmembrane potentials and metabolism of human fibroblasts

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ABSTRACT

Resting transmembrane potential (TMP) of primary human fibroblast cells was altered in predictable directions by subjecting cell cultures to specific monophasic and biphasic waveforms. Cells electrically stimulated with an anodal pulse resulted in hyperpolarization while a cathodal waveform depolarized the TMP to below that of non-paced control cells. The biphasic waveform, consisting of an anodal pulse followed immediately by an inverse symmetric cathodal pulse, also lessened the TMP similar to that of the cathodal pulse. The effect of short-term pacing on the TMP can last up to 4 h before the potentials equilibrate back to baseline. While subjecting the cells to this electrical field stimulation did not appear to damage the integrity of the cells, the three paced electrical stimulation waves inhibited expansion of the cultures when compared to non-paced control cells. With longer pacing treatments, elongation of the cells and electrotaxis towards the anodal polarity were observed. Pacing the fibroblasts also resulted in modest, yet very statistically significant (and likely underestimated) changes to cellular adenosine-5'-triphosphate (ATP) levels, and cells undergoing anodal and biphasic (anodal/cathodal) stimulation also exhibited altered mitochondrial morphology. These observations indicate an active role of electrical currents, especially with anodal content, in affecting cellular metabolism and function, and help explain accumulating evidence of cellular alterations and clinical outcomes in pacing of the heart and other tissues in general.
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1. Introduction

All live cells have a stable transmembrane potential (TMP) voltage differential across the cell membrane when the cell is at rest. This is the result of the accumulated ion concentrations within the cell compared to that outside the membrane. Electrically active cells, such as neurons, muscles, and pancreatic beta cells, are called excitable because they can produce an action potential due to a short-lived rapid depolarization of the TMP before returning to the higher resting state. These cells achieve this by expressing fast-acting voltage gated ion channels that allow the very rapid exchange of ions across the cellular membrane. While calcium and chloride ions make distinct contributions, it is particularly the sodium ($Na+$) and potassium ($K+$) which contribute most to this ion concentration potential.

Even cells that do not generate action potentials need to maintain a TMP in order to enable secondary active transport of metabolites. Non-excitable cells use slower membrane ion exchange pumps or transporters to move charges across the membrane, often in conjunction with the transport of a metabolite. Most of these transporters require a concentration gradient, a supply of energy, and are considerably slower than the fast-acting ion channels of the excitable cells.

Alterations to membrane potential are carefully modulated by ion channels within the cell membrane to maintain homeostasis. Subjecting cells to pacing waveforms alters the TMP since it results in a manipulation of these charged ions. We offer evidence that electrically altering the TMP can then have profound effects on cellular physiology in terms of both metabolism and function [1].

2. Materials and methods

2.1. Cells

Early passage human primary fibroblast cells (CRL-2703, ATCC; Manassas, VA) were subcultured in a 37 °C, high humidity

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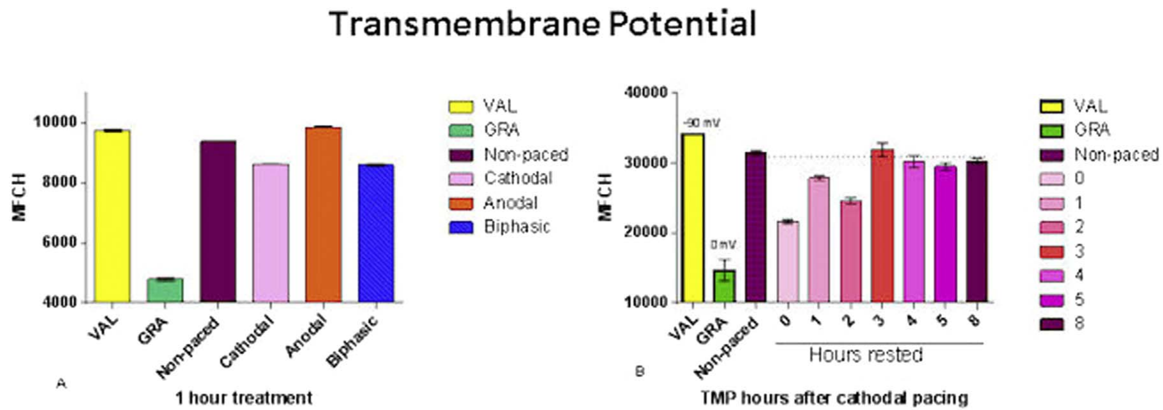

Fig. 1. Transmembrane potential measured with a cationic fluorescent dye after one hour of each treatment in panel A. Valinomycin caused hyperpolarization and gramicidin depolarization. Anodal pacing produced increased potential and cathodal and biphasic pacing showed less potential than unpaced cells. In panel B, the reduction of polarization caused by cathodal pacing required several hours to return to the baseline unpaced state.

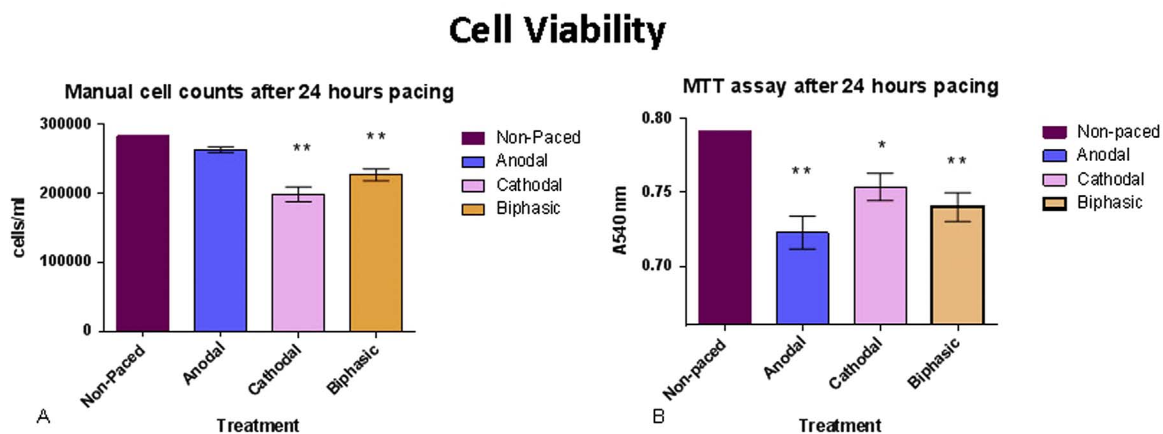

Fig. 2. In panel A, manual cell counts after 24 h of pacing showed inhibition of cell culture expansion with cathodal and biphasic waveforms, significant at p values less than 0.01 level. In panel B, MTT assay showed less cellular staining with any of the waveforms compared to non-paced at p values less than 0.01 (anodal and biphasic) and p value less than 0.05 level with cathodal.

incubator with 5% CO_2 and maintained in Iscove's Modified Dulbecco's Medium (IMDM, Thermofisher Scientific; Waltham, MA) supplemented with 10% fetal bovine serum (FBS; ATCC), 25 mM HEPES (Thermofisher Scientific) and an antibiotic-antimycotic solution (ATCC). Cells were passaged at approximately 80% confluence and checked weekly or biweekly to be mycoplasma-free. Twenty-four hours prior to pacing, cells were seeded at a density of $\sim 3.0 \times 10^5$ cells per cm^2 for attachment in designated pacing chambers.

2.2. Electrodes and pacing chambers

Pacing chambers were T-25 cell culture flasks, 6-well culture plates, and 3-D printed plastic forms with electrodes attached with similar spacing. Non-reactive carbon rods (4.0 mm diameter, Frey Scientific; Appleton, WI) or platinum wires (0.5 mm diameter, WPI; Sarasota, FL) were inserted 4 cm apart. There were no apparent variations of results in experiments based on the material composition of the electrodes. The chambers were tilted so that the seeded cells attached and were localized close to the active electrode, defined as the polarity of the defined waveform when measured on an oscilloscope. The other electrode served as the 'non-active' reference electrode.

2.3. Pacing treatment

An external cardiac pacer (PACE Medical, Inc.; Waltham, MA) set at 5 V (V) magnitude and 1.8 ms (mSec) corresponding to an output of 10 mA (mA) at a rate of 1.7 Hz was used as the input signal to a Slave Stimulator (Model 71006, Rivertek Medical Systems; Minneapolis, MN). This circuit produced the actual monophasic anodal and cathodal, and biphasic (anodal followed by cathodal) pulsed square waveforms used for stimulation. The monophasic waveforms selected for these experiments were ± 5.0 Vx1.8 ms. The biphasic waveform was defined as a $+2.5\ V \times 0.9$ ms anodal pulse immediately followed by a $-2.5\ V \times 0.9$ ms cathodal pulse. Selected waveforms were verified on the electrodes before each experiment using digital oscilloscopes with probes placed within the electrolyte near the active electrode. All pacing treatments were continuous for times stated with the cells incubated in the conditions described. Electrolyte for cells paced less than 24 h was the defined media; cells paced 24 h or longer included 5% FBS.

2.4. Transmembrane potential assay

After pacing treatment, cells were suspended in phenol red-free IMDM with 2% FBS and 25 nM DiOC6(3) and allowed to equilibrate to room temperature prior to cytometric analysis. The cationic potentiometric carbocyanine dye, 3,3′-dihexyloxacarbocyanine iodide (DiOC6(3), Thermofisher Scientific) was used to measure TMP on a flow cytometer (Beckton Dickenson Cantos, Japan) with BD FACSDiva software (Ver. 8.0). The positively charged dye is taken up by the cell in proportion to the negative

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