Investigation of the effects of aldosterone on the cardiac cycle using the HL-1 mouse atrial cardiomyocyte cell line

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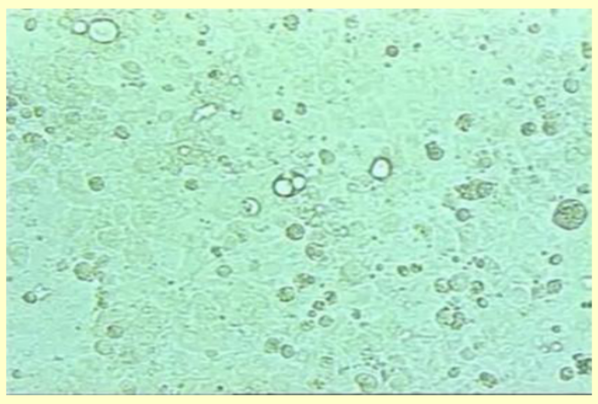
OBJECTIVES

In 1969, De Mello and Motta[1] reported that Aldosterone (Aldo) decreases the rate of repolarization in rabbit cardiomyocytes. Nowdays, it is widely known that Aldo decreases the cardiac output, causes acute input of Na^+ in vascular smooth muscle cells (VSMCs) and seriously affects the action of second messengers, such as Ca^{2+} and IP3. Tillman et al[2]., found a significant increase in the duration of monophasic action potential in patients with supraventricular arrhythmias in relation to Aldo levels, few minutes after intravenous administration, implying a non-genomic action. Kallaras et al[3], reported a positive linear regression between left ventricular contraction duration and plasma Aldo in NZW rabbits. To investigate the veracity and to elucidate the underlying mechanism of the above finding, we employ, in our Lab, the HL-1 cell line of mouse atrial cardiomyocytes.

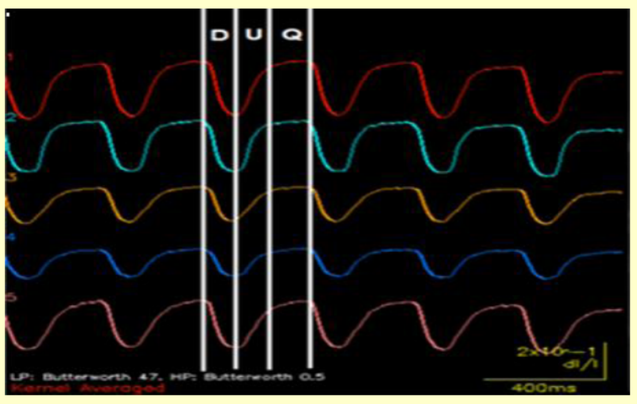
METHODS

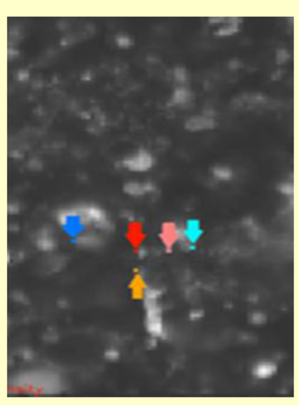
Confluent beating HL-1 cells were stained with 68μM di-8-ANEPS in 68μM Pluronic F-127. Cell beating and the accompanied fluorescent intensity changes, were recorded with a specialized high frequency sampling (1kHz), CMOS camera (NeuroCMOS-SM128f, Redshirt Imaging Inc, USA) coupled to a fluorescent microscope (AxioExaminer Z1, Carl Zeiss Microimaging GmbH, Germany). 15 optical recordings, of 2 sec duration, were taken 1 min apart. 50μM Aldo in Claycomb's Medium[4] was added between the 3rd and 4th recording, while the control received only the same volume of medium. Cardiac cycle total duration, contraction period (corresponding to cardiac systole) as well as relaxation period and duration of the "quite" state of the cells (both corresponding to cardiac diastole) were calculated.

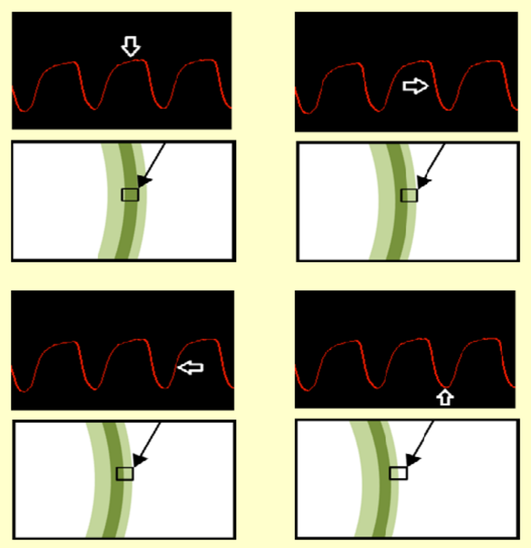
Graphs and tables



HL-1 cells in culture

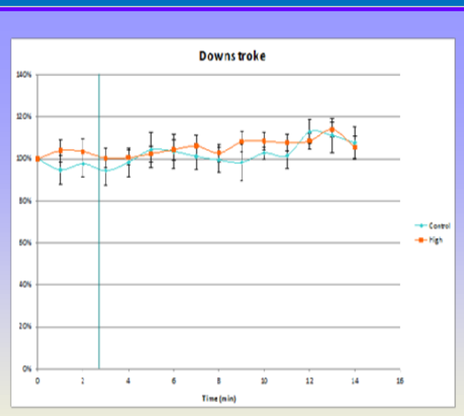




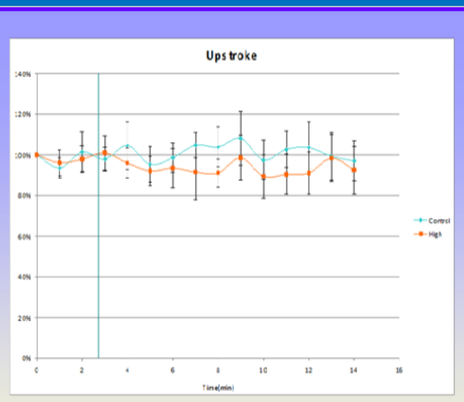


Recordings of the brightness change of different well points. It is clearly showed the periodic contraction of cells. Individual interrogation pixels at the right figure

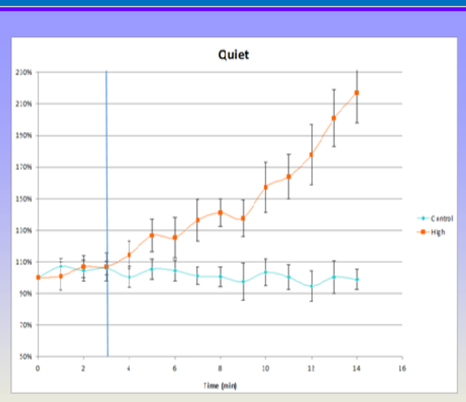
RESULTS



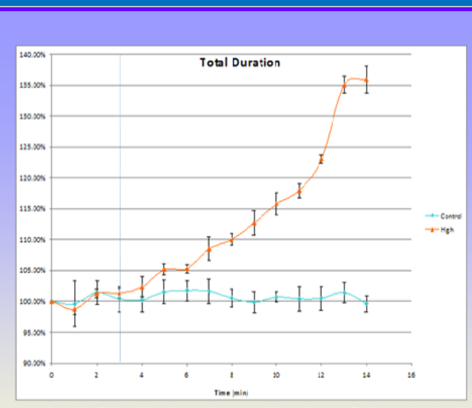
Graph 1. Statistical analysis of the Downstroke (systole) period's duration revealed no significant correlations



Graph 2. Statistical analysis of the Upstroke (diastole) period's duration revealed no significant correlations.



Graph 3. Statistical analysis of the Quiet period's duration revealed a SS increase after Aldo is added ($p<0.001$)



Graph 4.Statistical analysis of the total duration of the cardiac cycle revealed a SS increase after Aldo is added $p<0.001$)

The y-axis shows the percentage change of the variable. The blue vertical line represents the time when Aldo is added to the sample. Aldo increases the total duration of the cardiac cycle {$p<0.001$ [Z-Score (Standard Score), Spearman's Rho]}. This increase is attributed to the "quite" state of the cells corresponding to the diastolic phase of the cardiac cycle

CONCLUSIONS

Aldo increases the total duration of the cardiac cycle, decreasing thus the beating frequency. It does not affect the systole or relaxation times of the cardiac cell but the interval between them, relating to cardiac diastole.The previously found regression by Kallaras et al[3]. between systole duration (Δd) and Aldo levels may be justified since Δd contains the first part of cardiac diastole (protodiastole). Furthermore, it is important to report that the prompt response of the cells to Aldo denotes a non-genomic action. We persue with the investigation of the molecular basis of the observation with respect to Mineralocorticoid Receptor mediation and PKC involvement, as well as, to define the above results in response to the pathophysiology of heart failure.

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