Rubidium Uptake in Isolated Rat Cardiomyocytes

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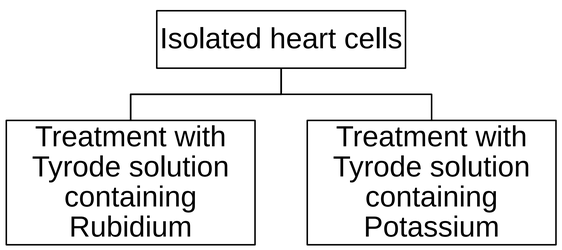
Introduction

Sodium and Potassium are essential elements for living tissues. The Na+/K+ ATPase pump maintains the intracellular and extracellular concentrations of potassium and sodium. Without adequate intracellular levels of potassium, the heart will not function normally. The element rubidium has similar properties to K+ and is transported by the Na+/K+ ATPase. From early research and studies, we know the cellular uptake of rubidium can be measured and localized with energy dispersive x-ray spectroscopy in conjunction with scanning electron microscopy (SEM) and can be used to examine the uptake of rubidium in cardiomyocytes.

Method

- The methods used are mainly three parts: 1) isolation of single heart cells, 2) treatment with rubidium, and 3) analysis with scanning electron microscope (SEM).
- Isolated heart cells were obtained by anesthetizing a rat, injecting heparin, extracting the heart, and infusing its aorta with cell isolation buffer (CIB) solution, and then a digestive enzyme solution containing collagenase, trypsin, and protease.
- The heart was cut into small pieces in the enzyme solution to further separate individual cells at 37° C.
- Once the cardiomyocytes were isolated, they were treated with Tyrode solution containing a physiological concentration of rubidium chloride.



- Both cell populations were washed with phosphate buffer solution (PBS) twice.
- Cells were frozen with liquid nitrogen and stored in - 80 ° C for 24 hours.
- Cells were freeze-dried for 72 hours and prepared for analysis.

Abstract

The element rubidium is known to have similar biological impact as K+ ions and some studies have shown that heart tissue takes up rubidium through K+ channels and Na+/K+ ATPase pumps. The purpose of the research was to confirm whether or not rubidium was transported into individual cardiomyocytes. Rubidium may be used as a marker to study different physiological functions of K+ and its channels and pumps. The methods used are mainly two parts: isolation of single heart cells and treatment with rubidium. Cells were freeze dried and examined using a scanning electron microscope (SEM) equipped with an Energy Dispersive X-Ray Spectroscopy System (EDS) to determine the amount of rubidium that was taken up by the cells. It is expected that there will be an uptake of rubidium cardiomyocytes by means of Na+/K+ ATPase pumps and K+ channels like K+. These ATPase pumps are essential in creation of concentration gradient and membrane potentials in most cells and K+ has many critical roles from muscle contraction to regulation of nerve conduction and propagation in heart cells.

Results

Fig.1

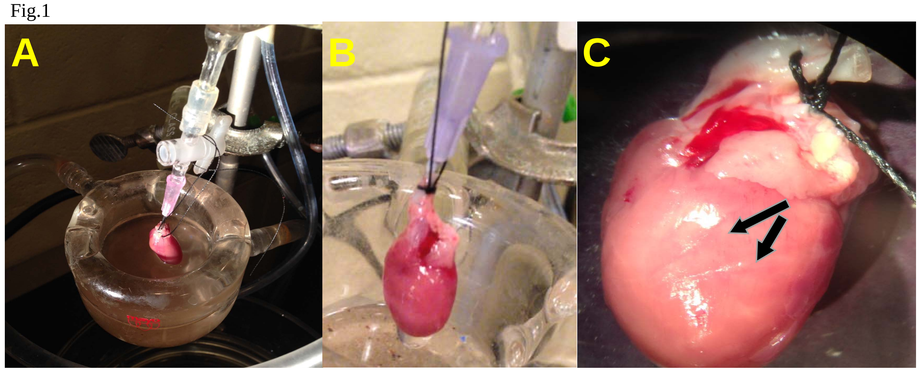
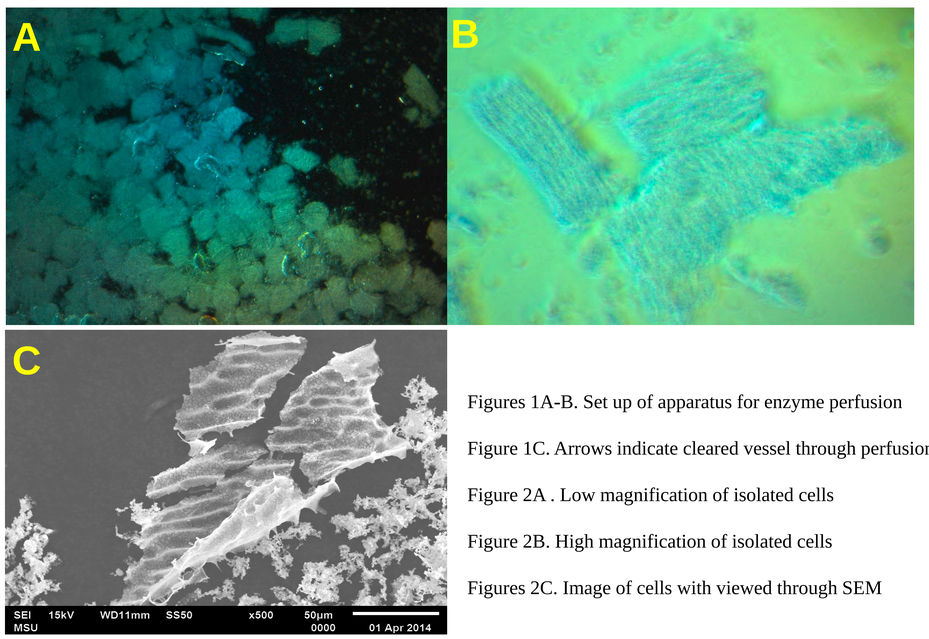


Fig2



Figures 1A-B. Set up of apparatus for enzyme perfusion

Figure 1C. Arrows indicate cleared vessel through perfusion

Figure 2A . Low magnification of isolated cells

Figure 2B. High magnification of isolated cells

Figures 2C. Image of cells with viewed through SEM

Conclusion

We have used enzyme solutions to isolate cardiomyoctyes(Fig.1A-C). We viewed the cell morphology using dark field light microscope (Fig. 2A-B) and SEM (Fig. 2C) . Preliminary results have shown the presence of rubidium in cells from hearts infused with Rb-solution but not in those from hearts infused with K-solution. Although we have detected the presence of Rb in the cells, we have not yet been able to obtain quantitative data because of time constraints.

Future Studies

- Obtain quantitative data and measure Rubidium uptake.
- Examine the Rb uptake at consecutive time intervals of treatment under SEM.
- Consider further diagnostics to quantify the intracellular Rb level.

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