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Ultracytochemical visualization of calcium distribution in heart cells and erythrocytes of zebrafish *Danio rerio*

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ABSTRACT

Detection of patterns of subcellular calcium distribution in the cardiovascular system can contribute to understanding its role in cardiac and blood function. The present study localized calcium in heart atrium, ventricle, and bulbus arteriosus as well as in erythrocytes of zebrafish *Danio rerio* using an oxalate-pyroantimonate technique combined with transmission electron microscopy. Intracellular calcium stores were detected in caveolae, mitochondria, and the nuclei of several zebrafish cardiac cell types. Melanin pigmentation containing calcium stores was detected in the pericardial cavity. Melanin might be an extracellular source of calcium for heart beating and/or a lubricant to prevent friction during beating process. Calcium deposits were also detected in the plasma membrane, cytoplasm and nucleus of erythrocytes as well as in blood plasma. Possible exchange of calcium between erythrocytes and blood plasma was observed. Interactions of such calcium stores and possible contribution of extracellular calcium stores such as melanin pigmentation to supply calcium for vital functions of heart cells should be addressed in future studies.

1. Introduction

Calcium plays important roles in contraction and relaxation of cardiac muscle throughout the animal kingdom (Shiels and Galli, 2014). Function of erythrocytes, including oxygen transport and blood coagulation, is highly dependent on calcium signaling pathways (Bogdanova et al., 2013). Therefore, characterization of extra- and intracellular calcium stores may contribute to better understanding of the role of calcium and related organelles in maintaining homeostasis.

The oxalate-pyroantimonate technique, in combination with transmission electron microscopy, has been utilized to study distribution of intracellular calcium in a variety of tissues (Ravindranath et al., 1994; Golpour et al., 2017). Such ultracytochemical techniques have been used for detection of subcellular calcium stores in the heart and erythrocytes of mammals (Borgers et al., 1983, 1984).

Zebrafish *Danio rerio* is increasingly used as a model organism in cardiovascular research (Shiels et al., 2009) and for the study of heart development and regeneration (Tu and Chi 2012; Itou et al., 2014). The zebrafish heart is comparable to humans in beating rate and electrophysiological properties (Werdich et al., 2012; Lin et al., 2015; Vornanen and Hassinen, 2016) and responds similarly to disease, toxins, and pharmaceuticals (Sehnert and Stainier, 2002; Tamaru et al., 2006; Dlugos and Rabin, 2010; Kim et al., 2013; Shurlock, 2014). A further advantage of zebrafish as a model is its size compared to adult mammals, allowing the entire heart to be analyzed on a single grid in transmission electron microscopy.

Atrium, ventricle, and bulbus arteriosus are three major compartments of the heart in zebrafish. The atrium is the upper chamber and conducts blood into the ventricle. The atrium and ventricle consist of similar cell types. The blood is pumped into the bulbus arteriosus in the anterior part of the zebrafish heart. The outer part of the zebrafish atrium and ventricle is covered by pericardium. The pericardium consists of epicardium that directly contacts the myocardium, the second layer of pericardium, and the pericardial cavity in between. The major part of the zebrafish atrium and ventricle is formed of myocardial cells. The inner layer of zebrafish atrium and ventricle consists of a single layer of endocardial cells. The bulbus arteriosus consists of smooth muscle cells and endothelial cells. The outer and inner layers of smooth muscle contain collagen and elastin, respectively. A single layer of endothelial cells formed the inner lining of the bulbus arteriosus (Hu et al., 2000, 2001; Menke et al., 2011).

Although the structure and ultrastructure of zebrafish heart is well described, information of intracellular calcium distribution in zebrafish is currently only available for gonads (Golpour et al., 2016a, 2017).

The objective of the present study was to characterize localization of calcium stores in the atrium, ventricle, and bulbus arteriosus in heart of

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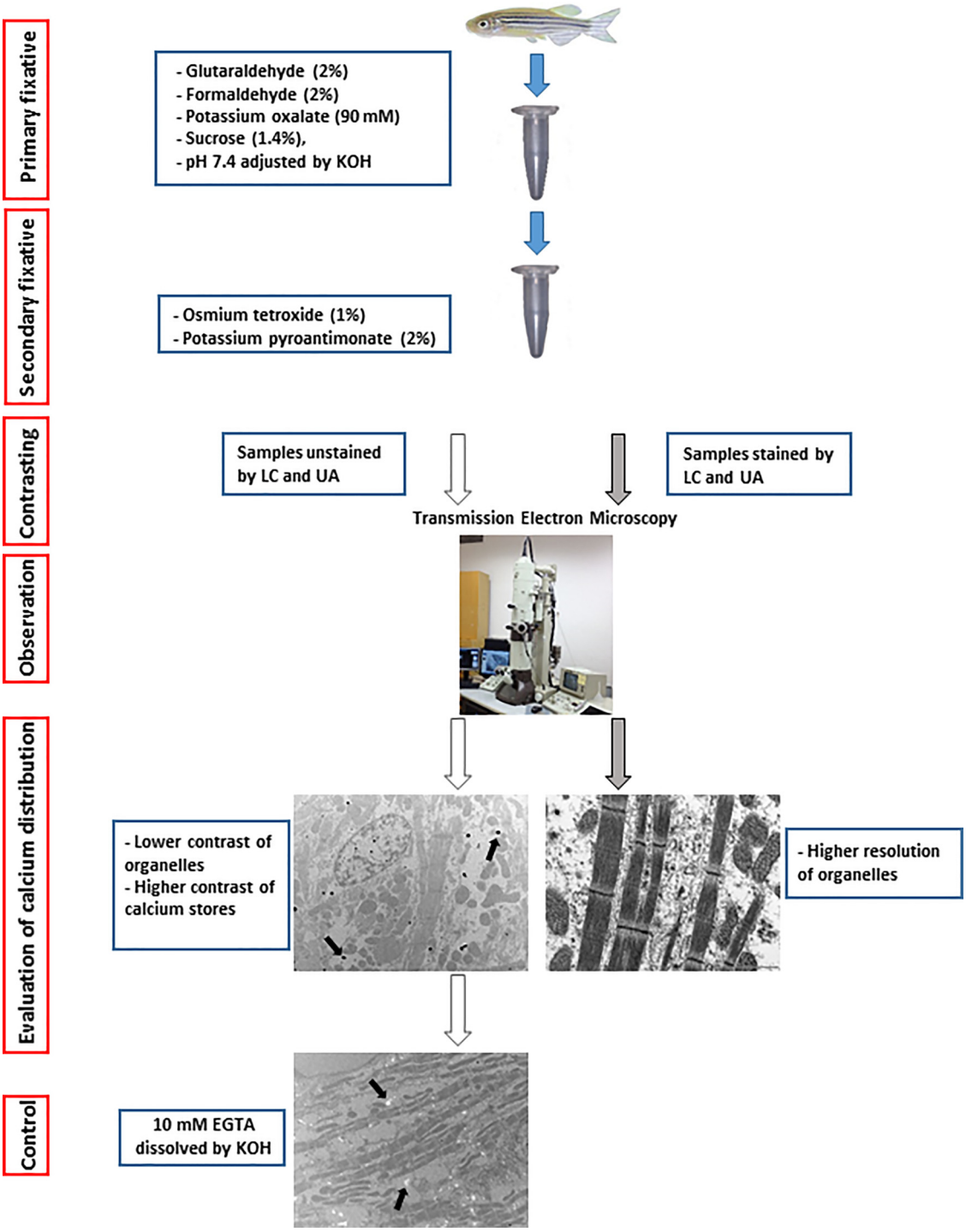

Fig. 1. Schematic workflow of the different stages of sample processing in oxalate-pyroantimonate technique. After primary and secondary fixations of intracellular calcium, samples are examined under TEM with and without contrasting by uranyl acetate (UA) and lead citrate (LC). In UA and LC stained samples, organelles show high contrast. The electron-dense calcium stores show high contrast and are distinguishable in non-stained samples, while contrast of organelles are lower. The results were interpreted using both forms of contrasted and non-contrasted micrographs. EGTA chelated calcium stores and left electron-lucent spaces on control micrographs.

zebrafish *Danio rerio* as well as in erythrocytes and blood plasma, using an oxalate-pyroantimonate cytochemical technique combined with transmission electron microscopy. Detection of patterns of subcellular calcium distribution in the cardiovascular system can contribute to understanding its role in cardiac and blood function.

2. Materials and methods

2.1. Animals

One-year-old *Danio rerio* (n = 6, wild type of the AB line, mean length 1.6 ± 0.2 cm) were obtained from the Department of Animal Science of the Brno University (Czech Republic). Twenty fish were placed in an aquarium containing 60 l fresh water with continuous