

Macula densa-dependent and non-macula  
densa-dependent signaling in the  
juxtaglomerular apparatus

PhD thesis outline

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## Introduction

The juxtaglomerular apparatus plays a pivotal role in the regulation of renal hemodynamics and also responsible for the production of renin, which is a major element of the renin-angiotensin-aldosterone system. A main element of the juxtaglomerular apparatus are the macula densa cells, which sense the changes in tubular salt concentration and osmolality and via releasing extracellular paracrine signaling molecules (ATP, prostaglandin E<sub>2</sub> and nitric oxide) affect the diameter of the adjacent afferent arteriole and the rate of renin release. The exact mechanism by which the macula densa cells sense the changes in tubular content is not known, but several functional studies suggested that changes in intracellular calcium concentration, pH, membrane potential and changes cell volume play a role. Interestingly, there are several conflicting reports about the direction and magnitude of cell volume changes upon increased distal tubular salt delivery.

Although it was never directly investigated it has been accepted that the macula densa plaque is the only

element in juxtaglomerular apparatus that senses the changes in tubular content. In the present studies we seek to describe the cell volume regulation of macula densa cells and to document the properties of a novel tubular epithelial cell type, the perimacular cells, which may play a role in the functioning of the juxtaglomerular apparatus.

The objectives of the dissertation are:

- 1. To establish a novel imaging model to study the juxtaglomerular apparatus in four dimensions at high temporal and spatial resolution.**
- 2. To assess the luminal NaCl and osmolality-dependent changes in macula densa and perimacular cell volume.**
- 3. To characterize the oscillations in intracellular  $\text{Ca}^{2+}$  concentration and membrane potential in perimacular cells. We will evaluate the effects of luminal NaCl concentration and luminal osmolality on the pattern of oscillations in intracellular  $\text{Ca}^{2+}$  concentration and membrane potential in these cells.**

**4. To determine the role of intracellular  $\text{Ca}^{2+}$  and membrane potential signaling in perimacular cells in the luminal sodium chloride concentration-dependent regulation of afferent arteriole activation.**

Methods

Superficial afferent arterioles with glomeruli and associated tubular segments containing the cTAL and DT were microdissected from rabbit kidneys and perfused *in vitro*. After transfer to a chamber that was mounted on the microscope, the arteriole and tubule was cannulated; the arteriole and tubule were perfused with bath and perfusion solutions, respectively. The preparations were loaded with various fluorescent dyes that allowed us to determine changes in cell volume, intracellular calcium concentration, membrane potential and to label cell membranes.

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*I dedicate my work to Bogi, Mom and Dad, my sons, Gergely and Dániel.*

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## Results

Physiologic increases in tubular salt concentration and osmolality lead to the shrinkage of macula densa cells. This change in cell volume is sustained, indicating that the water permeability of these cells is high and that they possess a limited ability to regulate their cell volume.

We have demonstrated that intracellular calcium concentration in the macula densa cells is lower than in the surrounding cells and the cells around the macula densa, the perimacular cells demonstrate spontaneous oscillations in intracellular calcium concentration and membrane potential. These cells produce specific intracellular calcium responses to changes in tubular salt concentration, osmolality and tubular flow. We also observed that the initial part of the distal tubule and the afferent arteriole establish a tight anatomical and functional connection.

## Summary

The juxtaglomerular apparatus plays an important role in the regulation of renal hemodynamics and in the control of renin release. Elevations in distal tubular salt delivery are sensed by the tubular epithelium (including the macula densa) which leads to the constriction of the adjacent afferent arteriole. In the present studies we utilized the isolated double perfused afferent arteriole-glomerular preparation with attached cortical thick ascending limb. To study changes in cell volume and the intracellular calcium concentration, basolateral membrane potential, we used multiphoton fluorescence microscopy and fluorescent dyes calcein, fluo-4, DiBAC<sub>4</sub>(3), respectively. We concluded that concomitant elevations in luminal sodium chloride concentration and osmolality produce macula densa cell shrinkage. This change in cell volume is maintained, suggesting the cells' limited ability to regulate their cell volume. The intracellular calcium concentration in the macula densa cells is relatively low and unresponsive to physiological challenges in the tubular lumen. On the other hand, cells in the vicinity of

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the plaque, called perimacular cells produce spontaneous oscillations in intracellular calcium concentration and basolateral membrane potential and produce characteristic changes in the pattern of intracellular calcium signaling. The early distal tubule and the adjacent afferent arteriole establish a close anatomical region of contact and functional relationship, suggesting that the perimacular cells and the connection of the early distal tubule and the afferent arteriole contribute to the paracrine signaling machinery of the juxtaglomerular apparatus.

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