Control of ureteral motility,
Synchronization of the circular and longitudinal muscle layers,
a novel videomicroscopic technique

Ph.D. Thesis
Fares Osman M.D.

Semmelweis University
Department of Urology
Clinical Experimental Research Department and Department of Human Physiology
Clinical Medicine Doctoral School

Program leader: Imre Romics M.D., Ph.D., D.M.Sc. Head of urological department
Tutor: Péter Nyirády M.D., Ph.D. Associate professor, University teacher

Scientific Referees of the Ph.D. Dissertation:
Zsolt Kelemen M.D., Ph.D., D.M.Sc. University teacher
András Kiss: M.D., Ph.D. University teacher

Chair of the Comprehensive Exam:
Emil Monos M.D., Ph.D., D.M.Sc. University teacher
Committee Members of the Comprehensive Exam:
Géza Bőszörményi-Nagy M.D., Ph.D. Head of urological department
Antal Hamvas M.D., Ph.D. University teacher

Budapest 2009
I. Introduction

Although the smooth muscle of the ureter is considered to be the active contributor in urine transportation, how its contraction is controlled and synchronized is far from sufficiently known. In the first part of this Ph.D. thesis we give a review of our present knowledge on ureteral smooth muscle contractility control processes. Most studies, done specially on ureteral smooth muscle have been listed. That analysis of existing literature encompasses practically all published material specifically on this topic. These literature data formed the material of a review paper published by us (Osman et al. 2009). In the second part of the thesis results of a series of own experiments will be shown in which we developed a new microsurgical-videomicroscopic technique to study the complicated motion pattern of the rat ureter. With the aid of this newly developed technique, first in the literature, a delicate analysis of the phases of ordered contractions of the circumferential and longitudinal layers of the ureter in vivo could be performed. As a result, a new interpretation of the physiological role of longitudinal and circular smooth muscle contractions could be given.

II. Aims of the study

Our first aim was to select all existing literature relevant for ureteral smooth muscle contractility control and analyze it. Our second aim was to establish a proper experimental technique that is able to study and evaluate the ureteral wall movement in vivo using videomicroscopy. In order to achieve this the ureter was exposed by micropreparation, then the middle portion of the ureter was isolated by installing it in a specially designed tissue chamber. This allowed the free movement of the ureter, while providing the possibility to check the composition of the surrounding fluid. Effects of drugs with known influence on ureteric activity could be applied locally and their effects tested. Using the videomicroscopic technique we developed enabled us to follow and analyze movements of designated surface points of the ureter. With careful mathematical analysis we were able to evaluate a new theory of the phases of contraction of different layers of the ureteral smooth muscle.
III. Methods and Materials

III.1. Surgery and tissue chamber

Male Sprague-Dawley rats weighing 250-350g were anesthetized and fixed on a temperature controlled operating table. The right carotid artery and the left jugular vein were cannulated to monitor blood pressure changes and to infuse drugs, respectively. The abdomen was opened with a midline incision. By careful micropreparation, the middle portion of the left ureter was cleared from the surrounding retroperitoneal fat tissue, while carefully sparing the blood vessels running along its surface. The relieved ureteral section was encased in a tissue chamber developed in our laboratory (Fig. 1). The chamber with its volume around 200 mm³ was perfused continuously at the rate of 5 ml/hour with warm oxygenized Krebs-Ringer solution using a continuous-flow pump (Braun). A continuous flow-withdrawal pump (Harvard Apparatus) set at the same rate as the flow pump was used to remove superfluous fluid.

Fig. 1. A three dimensional figure of the tissue chamber applied to study the rat ureter in vivo. 1. Superfusion inlet. 2. Superfusion outlet. 3. Groove for entrance of ureter. 4. Groove for exit of ureter. 5. Glass bottom. 6. Glass top. (According to Osman et al. 2009).
III.2. Data analysis

Digitized pictures were frozen at intervals of 166.7 msecs. Characteristic points were identified and their coordinates were recorded for further computations. The pattern of vessels running at the surface of the ureter makes it possible to identify the movements of some characteristic points as shown on (Fig. 2). Their coordinates were used to compute the movements of the ureteral wall in different directions.

![Diagram of ureteral peristalsis](image)

**Fig. 2. Studying ureteral peristalsis using videomicroscopy:** Three fixed points on the tissue chamber Cal1, Cal2 and Cal3 were used for position calibrations. Three cardinal points on the ureteral surface AV, BV and CV are marked by vasa vasorum network. The remaining six points A1, A2, B1, B2, C1 and C2 are edge points of ureteral contour at the levels of points AV, BV and CV, respectively. (According to Osman et al. 2009).

Movement of a characteristic point of the ureteral surface in the horizontal plane as a function of time is demonstrated using 3D Figures (Fig. 3). Outer diameter alterations at cross sections marked by characteristic points as a function of time represent mostly the activity of circular muscle. Axial shortening of a segment of the ureter between two characteristic points of the surface as a function of time represent mostly the activity of local longitudinal muscle. The axial movement of a characteristic point is determined by the activity of longitudinal muscle on a longer stretch of the ureter, either over or below the level
of videomicroscopic study. Further analysis of the periodic movements was made with applying autocorrelation functions (Fig. 4).

![Fig. 3. Trajectory of movements of the ureter cardinal point AV shown in (Fig. 2): a in radial and axial directions in the control state; b in radial and axial directions during systemic infusion of acetylcholine (0.83 g/min). (According to Osman et al. 2009).](image)

**IV. Results**

This method made it possible to follow the complex motion pattern of individual points of the ureteral surface during the periodic contractions. Three-dimensional plots (Fig. 3) revealed a characteristic motion pattern both in the axial and in the radial directions. Both frequency and amplitude altered upon systemic application of acetylcholine (Fig. 3 top and bottom records). The method provides possibility for an even more detailed analysis of ureteral movements. The periodicity of contractions could be further analyzed by computing autocorrelation functions. Figure 4 reveals the changes in ureteral motion pattern with systemic acetylcholine application. The basal periodic time was around 1.8sec which decreased close to 1sec with the acetylcholine infusion, but at the same time a more characteristic appearance of the 6.2sec periodic time component could be observed.
V. Discussion

With this technique, the longitudinal and circumferential contraction waves can be studied separately and their interaction analyzed. A detailed phase analysis of ureteral movements as measured in our experiments is shown in (Fig. 6). Active longitudinal contraction of a segment will induce a passive distention in the neighboring ones. We believe that this particular property of the ureter makes the longitudinal muscle contraction and its proper synchronization with the circular muscle contraction more significant. Based on our observations we suggest that for the analysis of the pathophysiology of ureteral diseases such as vesicoureteral reflux, ureteral obstruction and megaureter, moreover to be able to identify the effects of certain drugs on ureteral function, not only the frequency and amplitude of the circular muscle but the whole sequence of peristaltic contraction-relaxation cycles should be studied. The technique we described seems to be a promising one mostly for experimental purposes.
Fig. 5. **Suggested phases of the ureteral motion cycle:** Upper panels: diameter changes at points AV and CV, axial displacement of points AV and CV, and axial shortening between points AV and CV. Letters correspond to points shown in Fig. 2. Records were taken during systemic infusion of acetylcholine (0.83-g/min). Lower panel: 3D illustration of ureteral movements throughout its motion cycle as identified in the upper panel. Scattered red lines mark axial displacement of two characteristic points of ureteral contour. (According to Osman et al. 2009).
VI. Conclusions

The mechanism of ureteral movements as it can be revealed by using our experimental technique might give important new knowledge to understand the effects of drugs on the ureteral function as well as to understand pathological states affecting it. We developed a method that provides a technique to analyze the rat ureteral contractions with an accuracy that cannot be achieved using other available methods. Our preliminary observations revealed that the longitudinal smooth muscle contractions contribute to urine bolus propagation more effectively than it was thought earlier.

List of own Publications

   IF: 2.699
   IF: 0.491

Congresses abstracts related to the Thesis

the fifth European Urological Winter Escape Meeting. (An EU-ACME accredited conference)


