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

Ph.D. Thesis

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II. List of abbreviations

AC	adenylate cyclase
Ach-ase	acetylcholinesterase
ATP	adenosine 5’-triphosphate
cAMP	cyclic adenosine-3,5-monophosphate
cGMP	cyclic guanosine-3,5-monophosphate
CGRP	Calcitonin Gene-Related Peptide
CPA	cyclopiazonic acid
DAG	diacylglycerol
Forskolin	a PDE inhibitor
GC	guanylate cyclase
IP_3R	inositol trisphosphate receptor
MLC	myosin light chain
NE	noradrenaline
NO	nitric oxid
PKA	cAMP-dependent protein kinase
PKC	protein kinase C
PKG	cGMP-dependent protein kinase
SNP	sodium nitroprusside
SP/NKA	substance P and neurokinin A
SR	sarcoplasmic reticulum
RyR	ryanodine receptor
MLCK	myosin light chain kinase
NANC	non-adrenergic noncholinergic
NSAIDs	non-steroidal anti-inflammatory drugs
PDE	phosphodiesterase
PLC	phospholipase C
UPJ	ureteropelvic junction
Y-27632	a specific Rho kinase inhibitor

III. Introduction

The ureter is a muscular tube connecting the pelvis of the kidney with the bladder. There is no other way to dispose the urine produced by the kidneys, and there is no evidence of any change in the composition of urine as it traverses calyces, pelvis and ureter. The ureteral function can be considered as important a component of urine production as renal function itself. The coordinated muscular contraction propagating along the ureter provides the mechanism by which the function of the ureters is fulfilled. This myogenic activity has been termed ureteral peristalsis. The ureteral peristalsis myogenic theory can be traced back to Engelmann (1869) who was able to localize the peristaltic pressure wave's origin in the renal pelvis and suggested that the ureteral contraction impulse passes from one ureteral cell to another, the whole ureter working as a functional syncitium. The neurogenic contribution is thought to be limited to play a modulatory role in ureteral peristalsis.

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. Damage to ureteral urine transport is not an infrequent pathological event. Still, the number of works on the smooth muscle activity of the ureter either in health or in pathological states is limited. In the first part of this Ph.D. Thesis we will review our present knowledge on ureteral smooth muscle contractility control processes. Mostly studies, done specially on ureteral muscle will be listed. There will be only a limited usage of analogies with other types of smooth muscle, as despite existing similarities, the unique properties of the ureteral muscle render such automatic comparisons questionable. These literarure data formed the material of a review paper published by us Osman *et al.* 2009. In the second part of the thesis our own experiments will be shown in which we developed a new microsurgical-videomicroscopic technique to study the complicated motion pattern of the ureter, and with its careful phasic analyze a new interpretation of the longitudinal contractions could be given.

III.1. Ureteral wall structure

Before discussing the role of the ureteral physiology in the contraction relaxation cycle we need to understand the structure of the ureter, the histological features that enable the ureter to receive the urine produced by the kidneys to create a contraction and eventually to achieve its unique function transporting the urine bolus downward to the urinary bladder. The most interesting layer of the ureteral wall for us is the muscular layer because it is responsible for the ureteral motility, but the other layers such as the adventitia could not be neglected because our experiments included micro surgical preparation of the middle portion of the ureter and therefore the knowledge of the ureteral histology was essential to insure a successful preparation.

III.1.1. Rat ureter and renal pelvis

The upper urinary tract is a hollow structure which arises at the kidney capsule and terminates at the bladder. The ureter enters the kidney at the renal sinus and expands into a funnel-shaped organ, which is known as renal pelvis, which wraps around the renal papilla. In the dog, rat, guinea-pig, rabbit and other small mammals, the renal pelvis is unicalyceal. The most proximal regions of the renal pelvis form a number of finger-like septa, which serve as attachment points to the renal parenchyma. In these animals, urine is drained directly from the papilla into the renal pelvis, where it is then propelled into the ureter. In the human and the pig, the renal pelvis has a number of calyces (multicalyceal) into which urine drains. These minor calyces combine to form several major calyces that fuse to form the renal pelvis.

Ureteral smooth muscle function is among the least known physiological processes of the body despite its clinical significance. The reason is the hidden location of this organ; the ureter is located at a relative hidden place at the hind wall of the abdomen, the right ureter is 0.5 cm

inferior to the left ureter causing the right ureter to be few mm longer than the left ureter. Its small mass and complicated pattern of movements rendered other smooth muscles much easier for study. The subjects of our experiments were male Sprague–Dawley rats weighing 200–300 g (Fig. 1.). Due to the lack of the literature dealing with rat anatomy and failing to find any literature that was able to show us how to approach the rat ureter surgically we had to experiment with that and were able to establish a surgical procedure starting from our second attempt. The fact that the right ureter originates by 0.5 cm inferior to the left ureter led us to choose the rat's left ureter to be experimented rather than the right ureter.

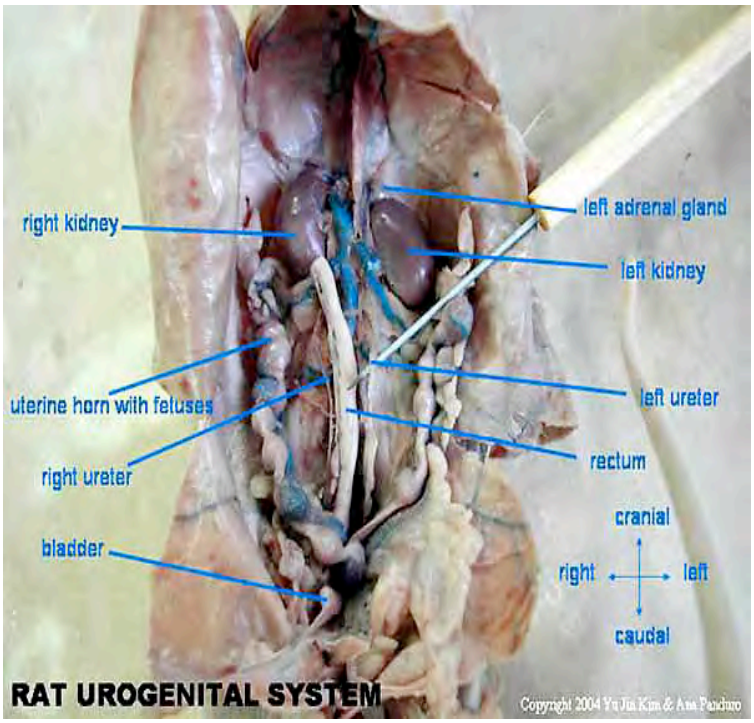

Figure 1. Anatomy of the rat upper urinary tract and neighboring organs of the rat. (According to Yu Jin Kim and Ana Panduro 2009)

III.1.2. Functional histology

The ureter in a 200-gm rat has an over-all diameter of approximately one third of a millimeter, or around 350 mμ, and a stellate-shaped lumen

varying between 75 and 150 mμ in diameter (Hicks 1965), in our experiments we catheterize the ureter using a plastic cannula with the diameter of 100 mμ avoiding the possibility of interfering with the ureteral peristaltic wave or the rupture of the ureter. The transitional epithelium lining the ureter is 3 to 4 cells deep and varies considerably in thickness, from 40 to 80 mμ, between the base and peak of the ridges. At the base of the epithelium, and in very close contact with it, are blood capillaries running parallel to the ureter lumen (Hicks 1965) (Fig. 2.).

About 50 of these axial capillaries may be counted in a complete cross-section of the ureter, and they connect at intervals through radial channels with the larger circumferential vessels which lie between the lamina propria and the outer muscle coat. The axial capillaries adjacent to the transitional epithelium have unusually thick endothelial cells with plentiful cytoplasmic contents. The lamina propria is composed mainly of collagen fibres and fibroblasts. This connective tissue layer supports the blood vessels, a few unmyelinated nerve fibres and, towards the periphery, longitudinal muscle cells. Peripheral to this there is a compact circular layer of smooth muscle, about 25 mμ thick, and the whole is surrounded by an adventitial connective tissue sheath carrying other blood vessels and nerve fibres (Hicks 1965) (Fig. 2.), in our experiments we spared those blood vessels because they are going to be of a great importance to later on be able to designate cardinal points on the surface of the ureter depending on the vasa vasorum pattern.

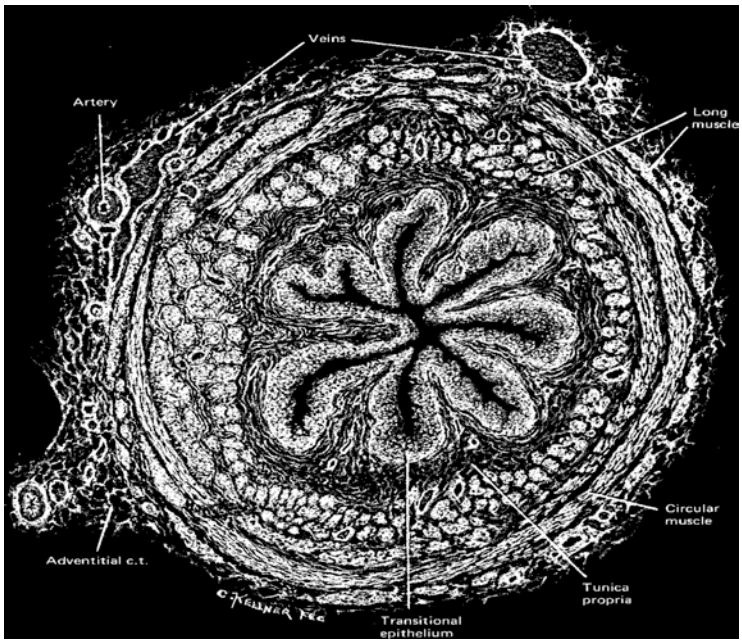

Figure 2. Tranverse section of contracted human ureter. Drawing is from lower part of the ureter and shows the addition of a discontinuous layer of outer longitudinal muscle. (According to Copenhaver WM and Johnson DD 1958)

III.1.2.1. The mucosa

The ureteral mucous membrane is composed of transitional epithelium, which is thought to be specialized to allow changes during the distention and contraction (Fig. 3.), due to the presence of a lubricant like intracellular material that enable the transitional epithelium cells to slide over each other (Chambers and De Renyi 1925). The superficial layer of the transitional epithelium serves as a protection against the urine acidity (Le Gros Clark 1958). In the contracted state, the ureteral mucous membrane, except in the pelvic part where it is thin, displays longitudinal folds, which give a stellate appearance to ureteral cross sections. Both transitional epithelium and longitudinal folds enable the ureter to stretch without rupturing during passing kidney stones. The lamina propria is a loose submucosal layer that varies in thickness, lies between the muscular coat and the transitional

epithelium. It consists of large amount of collagen fibres with accompanying fibrocytes and small vessels. Throughout the more superficial layers of the lamina propria are many bundles of unmyelinated nerve fibres (Fig. 2.).

III.1.2.2 The muscle coat

Ureteral contractions are formed by the contractions of the smooth muscle cells of the muscular layer (Fig. 2.). The upper third of the ureter has a thin muscular wall that consists of smooth muscle fibers that appear in small bundles and are separated by connective tissue. The middle third of the ureter has three muscular layers; a developed circular layer, an organized inner longitudinal layer, and a less well developed outer longitudinal layer. Satani's study (1919), using serial sections, revealed that both longitudinal coats show an increase in the amount of muscle fibers and in the size of each muscle bundle from the lower third, while the circular muscle reveals a decrease in size at the lower third. The lower third of the ureter is composed of a large number of longitudinal fibers and, some weak circular bundles.

III.1.2.3. The adventitia

The adventitia varies in thickness and is composed of areolar and fibroelastic connective tissue (Fig. 2.). The lowermost aspect of the human ureter, for a distance of 3 to 4 cm, contains a specialized fibrous tissue cover possessing a group of longitudinal muscle fibers located on one side, known as Waldeyer's sheath (Waldeyer 1892). The space between Waldeyer’s sheath and the ureteral wall contain some loose connective tissue, which serves as a lubricant to facilitate the slight movement between the ureteral lower end and the vesical wall during the contraction and relaxation of the bladder, and has for its chief purpose the prevention of regurgitation urine (Wood Jones 1953).

III.1.3. The contractile apparatus

Regarding the contractile apparatus of the ureter we encountered the problem of the limited number of publications that are specifically handling the ureteral contractile apparatus. Scattered observations mostly on pathological specimens gave us the impression that the ureteral smooth muscle contractile apparatus is similar to or identical with that of other smooth muscle but a detailed, targeted analysis is missing. Similarly to other smooth muscle, the cytoskeleton of ureteral smooth muscle cells is composed of three major filamentous systems. Microfilaments are thin structures formed of G-actin subunits; its network is responsible for the gel consistency of the marginal cytoplasm (Condeelis J, 1981). After interaction with the head fragment of myosin, microfilaments form arrowheads (Ishikawa H, 1969). Some of the actin modulators regulate the rigidity of cytoplasmic gel, formed by F-actin and other gelation factors. Microtubules are hollow, noncontractile structures. They can quickly disassemble into subunits (tubulin dimers), leading to a dynamic cellular scaffold rather than a rigid skeleton. Microtubules are sensitive to high hydrostatic pressure, low temperature, and high concentrations of calcium, which plays an important role in regulating the assembly. Microtubules are often bound to specific proteins (MAPs) that mediate interaction between actin filaments and microtubules, leading to a network consisting of microtubules cross-linking microfilaments (Griffith LM, 1978; Sattilaro RF, 1981). Intermediate filaments show a tendency to associate with other cellular structures, such as microtubules, membranes (Ramaekers FCS, 1982), polyribosomes, and specific proteins (Linder E, 1979; Zumbe A, 1982). They are insoluble under physiological conditions, which indicates that they are biochemically related. Intermediate filaments are immunologically related and share antigenic determinants (Wang C, 1980; Pruss RM, 1980).

III.2. Ureteral peristalsis

The ureteral function is to transport urine to the bladder adaptively. The coordinated muscular contractions that propagate along the ureter provide the mechanism by which this function is discharged, this muscular activity has been termed ureteral peristalsis. Ureteral peristaltic contractions in vivo are affected moderately by parasympathetic and sympathetic blockers (Golenhofen and Hannappel, 1973). Whereas, the sensory nerve function blockers or prostaglandin production reduces the peristalsis in vitro (Davidson and Lang, 2000; Lang *et al.,* 2002). Ureteral spontaneous contractions occur in vivo after denervation, after auto transplantation (Santicioli and Maggi, 1998), and in vitro if the renal pelvis remains attached (Lang *et al.,* 2001; Davidson and Lang, 2000; Teele and Lang, 1998).

Ureteral pressure recordings in vivo reveal that both the amplitude and frequency of ureteral peristalsis are affected by the urine volume. During periods of low urine production only few muscle contractions travel along the ureter. Higher rates of diuresis, improves transmission from the renal pelvis to the point that a one-to-one propagation of contractions to the ureter is created (Constantinou and Hrynczuk, 1976; Constantinou *et al.,* 1974). Thus the initiation and control of ureteral peristalsis is considered to be myogenic in nature, being dependent on the activity of a pacemaker region in the renal pelvis under the influence of, but not triggered by, the rate of urine flow.

In both uni- and multicalyceal kidneys, a single pacemaker region on the pelvi-calyceal border is responsible for every wave of activation which conducts radially across the pelvis to form a wave that conducts distally towards the UPJ (Lammers *et al.,* 1996; Shimizu, 1978; Yamaguchi and Constantinou, 1989). Circumferentially cut strips of muscle wall, dissected from different regions equally distant from the papilla base, contract at the same frequency (Constantinou. and Yamaguchi, 1981; Yamaguchi and Constantinou, 1989). In contrast, contraction frequency decreases as strips are dissected from sites more distant from the papilla base (Lang *et al.,* 1998;

Lang *et al.*, 2002; Potjer *et al.*, 1992; Patacchini *et al.*, 1998). The ureter in the multipapillate kidney contracts spontaneously in vitro; while spontaneous contractility in the ureter of uni-papillate kidneys is only observed in vitro if the proximal renal pelvis remains connected (Hannappel and Golenhofen, 1974; Davidson and Lang, 2000; Teele and Lang, 1998).

Dwyer and Schmidt-Nielsen (2003) suggested that contractions of the muscle wall at the papilla base of the hamster kidney creates oscillating hydrostatic and osmotic gradients that are essential in the initial emptying of the papilla and the movement of fluids through the loops of Henle and collecting ducts. Osman *et al.* (2008) studied the rat ureteral peristalsis using a novel videomicroscopic technique and separated a four-phase cycle of ureteral contraction. These studies revealed that besides the known function of circular muscle in the propagation of the urine bolus, the longitudinal smooth muscle also plays an essential role. Longitudinal contraction spreading downward axially distends passively the uppermost portion of the ureter forming a "ureteral diastole" that helps filling. Oppositely, the longitudinal contraction of the lower portion, followed by the circular contraction ring, helps injecting urine into the bladder (Fig. 3.)

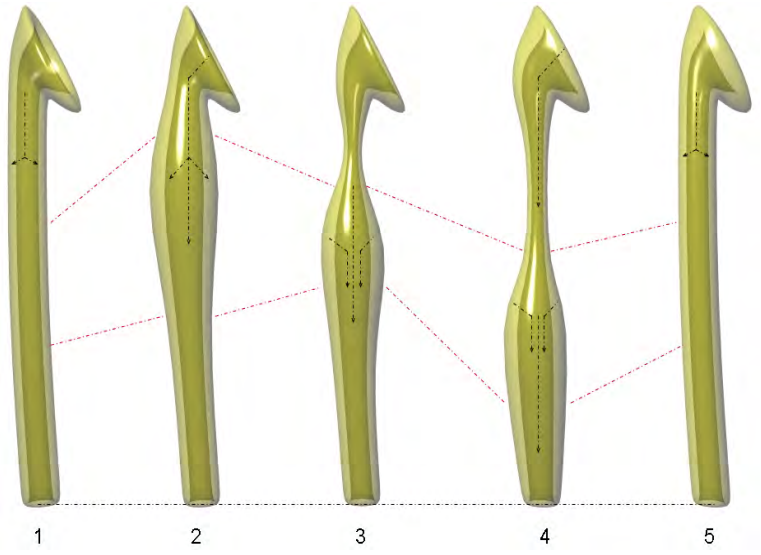

Figure 3. Suggested phases of the ureteral motion cycle. 3D illustration of ureteral movements throughout its motion cycle as identified in the upper panel. Scattered red lines mark axial displacement of two characteristic points of ureteral contour. (According to Osman *et al.* 2009a)

III.3. Regulating factors of the ureteral peristalsis

It has been established that the basic process regulating ureteral peristalsis is myogenic, ureteral peristalsis is the product of the ureteral smooth muscle layer contraction. To better understand the myogenic process we need to understand the factors that regulate the contraction relaxation cycle of the ureteral smooth muscle. Intracellular Ca^{2+} plays a central role in contraction control. Rhythmic contractions of the ureter are driven by propagating membrane depolarizations. Part of calcium enters the cells through voltage sensitive calcium channels, the effect of voltage alterations will be discussed later in chapter excitation/contraction coupling. Additional cellular regulation of force and frequency has been identified through intracellular levels of cyclic adenosine-3,5-monophosphate, cyclic guanosine-3,5-monophosphate, inositol/triphosphate, the activity of the Rho-kinase and tissue prostanoids. Their effect will be discussed in this chapter.

III.3.1. Role of cAMP, cGMP

Cyclic adenosine-3,5-monophosphate (cAMP) and cyclic guanosine-3,5-monophosphate (cGMP) are intracellular second messengers which mediate cellular responses and are involved in the relaxation of smooth muscle cells Pozzan *et al.* (1994). Cyclic nucleotides are synthesized from their corresponding nucleoside triphosphate by adenylyl cyclase and guanylyl cyclase respectively (Fig. 4.), and are degraded by phosphodiesterase (a heterogenous group of hydrolytic enzymes), through cleavage of the 3'-ribose phosphate bond. An increase in cAMP and/or cGMP triggers a signal transduction cascade encompassing the activation of cyclic nucleotide-dependent protein kinases, inducing subsequent phosphorylation of membrane Ca^{2+} channels. This cascade leads to a reduction in cytosolic Ca^{2+} and, finally, smooth muscle relaxation (Schmidt *et al.*, 1993) (Fig. 4.).

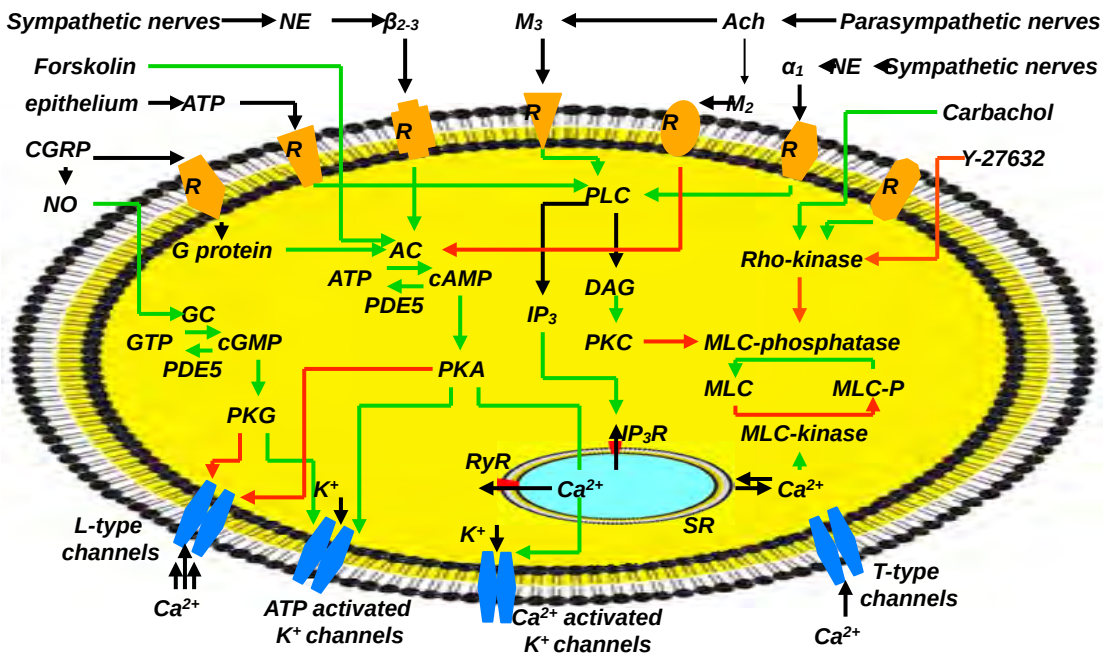

Figure 4. Schematic depiction of some of the physiological-pharmacological basis for ureteral contraction and relaxation. R, each represent different type of receptor; SR, sarcoplasmic reticulum; RyR, ryanodine receptor; IP_3R, inositol trisphosphate receptor, ATP, adenosine 5'-triphosphate; AC, adenylate cyclase; GC, guanylate cyclase; cAMP, cyclic adenosine-3,5-monophosphate; cGMP, cyclic guanosine-3,5-monophosphate; NO, nitric oxide; PDE, phosphodiesterase; Forskolin, a PDE inhibitor; PLC, phospholipase C; DAG, diacylglycerol; PKC, protein kinase C; MLC, myosin light chain; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; CGRP, Calcitonin Gene-Related Peptide; Y-27632, a specific Rho kinase inhibitor; NE, noradrenaline; α_1, α_1-adrenoceptor; β_{2-3}, β_2, and β_3, adrenoceptor subtypes; Ach-ase, acetylcholinesterase; M_2 and M_3, muscarinic receptor subtypes. Green arrows are the stimulatory pathways, while red arrows represent inhibitory regulation. (According to Osman *et al.* 2009b)

Cyclic nucleotide phosphodiesterase (PDE) isoenzymes are considered to be key proteins in regulating the intracellular cyclic nucleotide turnover and thus smooth muscle tension (Pozzan *et al.*, 1994; Stief *et al.*, 1996). The

cyclic nucleotide turnover is low in isolated tissue preparations, whereas, its turnover rates are much higher in the in vivo systems where PDE inhibitors tend to be much more effective (Nicholson *et al.*, 1991). Therefore, Kuhn *et al*. (2000) suggested that future studies on ureteral smooth muscle relaxation and determination of cyclic nucleotide levels would gain importance under better physiological conditions, i.e., by addition of sub-saturating concentrations of forskolin or sodium nitroprusside SNP and various concentrations of PDE inhibitors (Fig. 5,6.).

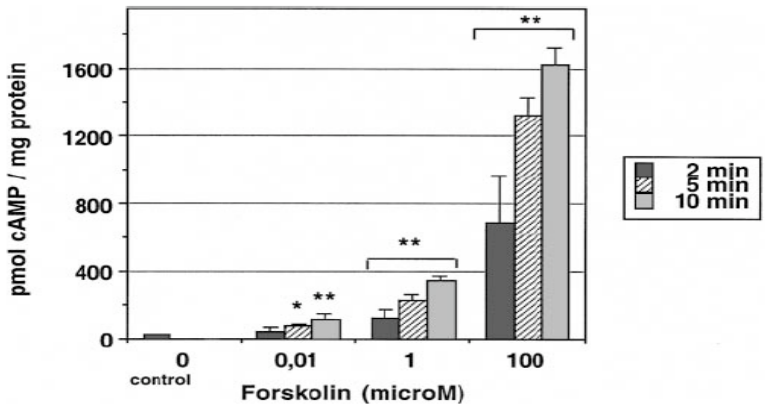

Figure 5. Time course of cAMP accumulation in the presence of forskolin in human ureteral segments. Each bar represents the mean. SD of three individual determinations. *Values significantly different from control (*P < 0.05, **P < 0.01). (According to Kuhn *et al*. 2000)

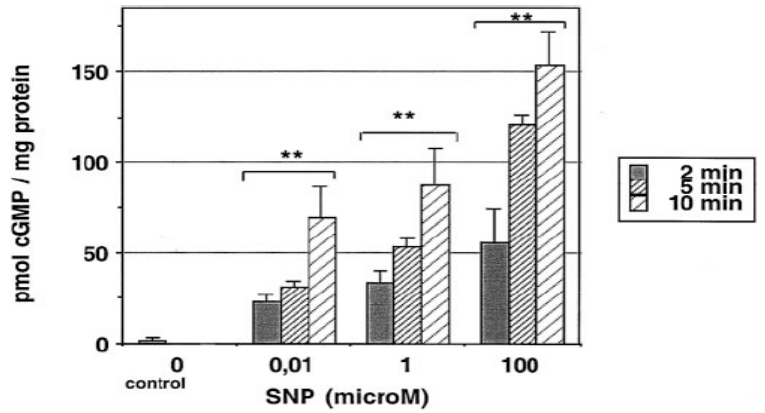

Figure 6. Time course of cGMP accumulation in the presence of SNP in human ureteral segments. Each bar represents the mean. SD of three individual determinations. *Values significantly different from control (*P < 0.05, **P < 0.01). (According to Kuhn *et al*. 2000)

III.3.2. Role of Rho-Kinase

The increase in cytoplasmic free Ca^{2+} concentration is regarded to be the primary mechanism in smooth muscle contraction (Ganitkevich and Isenberg, 1995; Taggart and Wray, 1998) however, other secondary mechanisms can modulate smooth muscle contractility such as Rho-kinase which signaling mechanism was reported to play a major role in the contraction process (Somlyo and Somlyo, 1994-1998). Studies have shown a role for the Rho-kinase pathway in ureteral contractions (Shabir *et al.*, 2004; Levent and Buyukafsar, 2004; Hong *et al.*, 2005) (Fig. 4.). Activation of Rho-kinase inhibits smooth muscle myosin phosphatase by phosphorylating its regulatory subunit, which in turn prevents the dephosphorylation of myosin light chain, leading to Ca^{2+} sensitization of the smooth muscle (Hong *et al.*, 2005; Somlyo and Somlyo, 2003) (Fig. 4.). Therefore, inhibition of Rho-kinase may result in smooth muscle relaxation. Y-27632, a specific Rho kinase inhibitor (Ishizaki *et al.*, 2000), relaxes animal and human ureteral smooth muscle (Levent and Buyukafsar, 2004; Hong *et al.*, 2005) (Fig. 7.). Studies have shown that Y-27632, by inhibiting Rho-kinase, can decrease light-chain phosphorylation (Shabir *et al.*, 2004; Sward *et al.*, 2000; Miyazaki *et al.*, 2002; Oh *et al.*, 2003) (Fig. 4.). Unexpected effects of Y-27632 on the action potential were found in rat ureter, Y-27632 markedly reduced the duration of the plateau component of the action potential evoked by electrical stimulation or carbachol, reduced Ca^{2+} signals and hence had deleterious effects on force production (Shabir *et al.*, 2004). Shabir *et al.* (2004) suggested that the effect of Y-27632 is caused by the decrease in Ca^{2+} due to the partial inhibition of the voltage-gated Ca^{2+} channels. Restoration of the action potential by Bay K8644 or TEA could overcome the effects of Y-27632 on the Ca^{2+} transient and restore force. These data are consistent with findings that Bay K8644 reverses the effects of Y-27632, moreover suggest that plasma membrane Ca^{2+} channels can be targets for Rho-kinase (Shabir *et al.*, 2004).

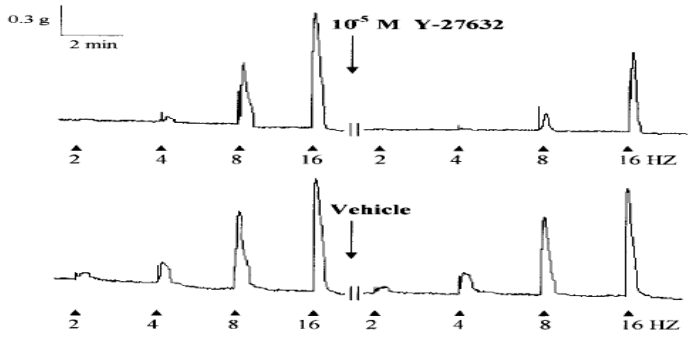

Figure 7. Tracings showing the effects of Y-27632, a Rho-kinase inhibitor and its vehicle (distilled water, 0.1–10ml organ bath) on the electrically (40 V, 1mS, for 20 s) induced contractile activity in the isolated whole vas deferens of mouse. After the first series of responses, Y-27632 or the vehicle was incubated for 30 min with the vas deferens before the second series was obtained. Note that both phasic and tonic contractile responses were attenuated in the presence of Y-27632. (According to Buyukafsar *et al.* 2003).

III.3.3. Role of Prostanoids

Prostaglandins play an important role in regulating many different biological processes such as homeostasis, modulation of kidney and gastric function, inflammation and the maintenance of smooth muscle contractility. The application of prostaglandins can have either an excitatory or inhibitory action on the smooth muscle contractility of the upper urinary tract, depending on the type and concentration of the prostanoid, and the tissue and species involved (Johns and Wooster, 1975; Karmazyn and Dhalla, 1983). Boyarsky and colleagues demonstrated that the exogenous application of PGE1 inhibited the spontaneous contractions in the dog ureter in vitro and in vivo (Boyarsky *et al.* 1966). In human ureteric preparations, both PGE1 and PGE2 decreased the spontaneous contractions, while PGF_{2a} increased muscle contractility (Abrams and Feneley, 1976). A summary of the effects of exogenous prostaglandins on spontaneous or evoked contractions in the renal pelvis and ureter of the guineapig, dog, rabbit, human and sheep is presented in (Table. 1.). In general, PGF2a increases evoked or spontaneous

contractility of the upper urinary tract (Abrams and Feneley, 1976; Angelo-Khattar *et al.,* 1985; Cole *et al.*, 1988; Zhang and Lang, 1994) while PGE1 decreases contractility (Johns and Wooster, 1975; Michibayashi, 1978; Karmazyn and Dhalla, 1983; Vermue and Den Hertog, 1987). The effects of applied PGE2 are more variable. Researchers have reported a decrease (Abrams and Feneley, 1976; Vermue *et al.*, 1987; Vermue and Den Hertog, 1987), an increase (Lundstam *et al.* 1985; Angelo-Khattar *et al.* 1985; Thulesius and Angelo-Khattar, 1985; Cole *et al.*, 1988) and no change (Andersson and Forman, 1978) in ureteric contractility upon exposure to PGE2 (Table .1.).

Table 1. Effects of prostaglandins $PGF_{2\alpha}$, PGE_1 and PGE_2 on pyeloureteric contractility

Prostaglandin	Species	Preparation	Effect	Reference
$PGF_{2\alpha}$	Human	Renal pelvis	Excitation	Zwergel *et al.* (1990)
			Excitation	Angelo-Khattar *et al.* (1985)
		Ureter	Excitation	Abrams & Feneley (1975)
			No change	Forman *et al.* (1978)
	Guinea-pig	Renal pelvis	Excitation	Zhang & Lang (1994)
		Ureter	No effect	Vermue & Den Hertog (1987)
	Rabbit	Ureter	Excitation in indomethacin	Lundstam *et al.* (1985)
	Sheep	Ureter	Excitatory	Thulesius & Angelo-Khattar (1985) Thulesius *et al.* (1986)
PGE_1	Human	Ureter	Inhibition	Abrams & Feneley (1975)
			No effect	Forman *et al.* (1978)
	Guinea-pig	Ureter	Inhibition	Johns & Wooster (1975) Michibayashi (1978)
	Dog	Ureter	Inhibition	Boyarsky *et al.* (1966)
PGE_2	Human	Ureter	Excitation	Angelo-Khattar *et al.* (1985)
			No effect	Forman *et al.* (1978)
			Inhibition	Abrams & Feneley (1975)
	Guinea-pig	Ureter	Inhibition	Vermue & Den Hertog (1987)
	Rabbit	Ureter	Excitation in indomethacin	Lundstam *et al.* (1985)
	Sheep	Ureter	Excitation	Thulesius & Angelo-Khattar (1985)

Prostaglandins play an important role in the maintenance of normal physiological function of the upper urinary tract. In addition to a possible central analgesic effect, a reduction of renal blood flow and a reduced urine production are considered to be major mechanisms through which the cyclo-oxygenase enzyme inhibitors (COX is the first, rate limiting enzyme in prostaglandin production) produce pain relief in renal colic (Perlmutter *et al.*, 1993). There are two isoforms of COX: COX-1 that mediates the normal

physiological functions, while COX-2 is responsible for mediating the inflammatory responses. Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit the synthesis of renal prostaglandins. Mastrangelo *et al.* (2000) studied the influence of the non-selective (COX) inhibitor diclofenac, a NSAID drug used in the treatment of renal colic, and of NS-398, a selective COX-2 inhibitor, on induced contractions of the pig ureter. Similarly, celecoxib (a selective COX-2 inhibitor) and indomethacin (a non-selective COX inhibitor) both inhibit PG release in the ureter even in the presence of COX-2 induction (Jerde *et al.,* 2005). Zhang and Lang (1994) showed that indomethacin inhibits the amplitude and frequency of action potentials from “driven” cells in the guinea pig proximal renal pelvis and eventually caused a failure of driven cells to fire action potentials.

III.3.4. Role of sensory nerves

There is considerable evidence that capsaicin-sensitive sensory neurons play a role in maintaining contractility in the mammalian upper urinary tract. In primary afferent nerves distributing to the mammalian renal pelvis and ureter two tachykinins (substance P and neurokinin A) with an established status of neurotransmitters are present. CGRP also coexists with SP/NKA in many sensory nerves; CGRP positive (CGRP1) nerves exist in the ureter that do not colocalize with SP/NKA (Sann *et al.,* 1992; Zheng and Lawson, 1997). Due to the sub- and intraurothelial distribution of the SP/NKA/CGRP1 nerves (Zheng and Lawson, 1997; Tamaki *et al.,* 1992), the sensory nerves are able to detect a back-flow of urine into the renal pelvis and ureteral wall. The density of fibers penetrating the urothelium seems larger in the renal pelvis than in the ureter (Zheng and Lawson, 1997). The distribution of SP/NKA/CGRP1 nerve fibers to the mammalian ureter has been detailed in several studies (Tamaki *et al.,* 1992; Zheng and Lawson, 1997). In the renal pelvis, the fibers run parallel to the long axis of each of the circular and longitudinal muscle layers. In the ureter, the fibers accumulate in the subepithelial plexus, around blood vessels, and in the muscle layer. In the human ureter SP/CGRP1 nerves

are mostly present around blood vessels and in the ureteral submucosa but are occasionally seen in the smooth muscle layer, and are of a lower density than those in other species.

Santicioli and Maggi (1994) showed that CGRP produces a glibenclamide-sensitive hyper polarization of the guinea pig ureteral smooth muscle, which resembles the hyper polarizing action of cromakalim (Maggi *et al.*, 1994). In the presence of glibenclamide, CGRP shortened the action potential duration and reduced the amplitude of the accompanying contraction (Fig. 8.). Santicioli *et al.* (1995) showed that while CGRP has no effect on cGMP it determines the cAMP accumulation in the guinea pig ureter. Forskolin was found to be more effective in elevating cAMP than CGRP since it activates adenylyl cyclase directly, while the CGRP action is mediated by a G-protein coupled to CGRP receptors (Santicioli *et al.*, 1995) (Fig. 4.). NO/ cGMP pathway is not responsible for the CGRP relaxant activity because CGRP do not elevate cGMP levels in the ureter (Santicioli *et al.*, 1995) (Fig. 4.).

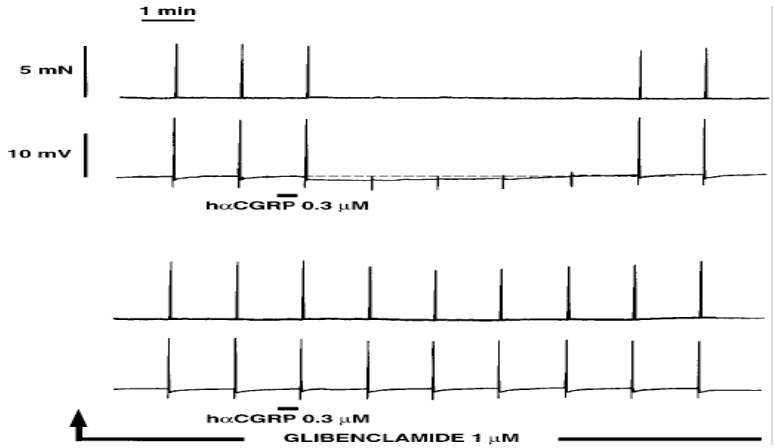

Figure 8. Action potential (recorded by sucrose gap technique) and phasic contraction produced by electrical stimulation of the guinea-pig ureter: note the hyper polarization and transient suppression of evoked electrical and mechanical responses produced by CGRP (0.3 mμ for 15 sec, applied at bars) and antagonism of the action of CGRP by glibenclamide. Note that in presence of glibenclamide CGRP produced a small inhibition of the amplitude of evoked contractions. (According to Santicioli P and Maggi CA 1998).

III.4. Excitation-contraction coupling

Action potentials spreading to the membrane of ureteral smooth muscle cells will induce their contraction through mechanisms common also for other smooth muscle type. The major mechanism responsible for the increase in mechanical force in smooth muscles and as a consequence activating contraction is the rise in intracellular Ca^{2+} (Ganitkevich and Isenberg, 1995; Taggart and Wray, 1998).

Activation of muscarinic acetylcholine receptors enhance the ureteral contraction through activating phospholipase C (PLC), which in turn causes the formation of the second messengers inositol trisphosphate (IP_3) and diacylglycerol (DAG) (Berridge, 1984) (Fig. 4.). IP_3 activates Ca^{2+} mobilization from sarcoplasmic reticulum (Streb *et al.*,1983), whereas DAG increases calcium influx across the cell membrane through activating protein kinase C (Nishizuka 1984). Stimulation of muscarinic receptors by carbachol (a muscarinic agonist) causes contraction of the pig isolated intravesical ureter (Hernandez 1995). However, Roshani *et al.* (2003) demonstrated that smooth muscle activity in the middle and distal portion of the ureter was not modulated by muscarinic receptors in porcine preparations. In another study, cholinergic receptor stimulation by carbachol in anesthetized dogs had a suppressive effect on ureteral pressure and peristalsis in obstructed ureters Tomiyama *et al.* (2004).

The source of Ca^{2+} enters the cell across the membrane surface is the sarcoplasmic reticulum (SR). There are two mechanisms responsible for the Ca^{2+} release from the SR, either by an IP_3-induced Ca^{2+} release mechanism, or by a Ca^{2+}-induced Ca^{2+} release mechanism (Somlyo and Somlyo, 1994) (Fig. 4.). Many smooth muscles use both mechanisms but some uses only one of them, i.e. the guinea-pig ureter has a purely Ca^{2+}-induced Ca^{2+} release mechanism (Burdyga *et al.*, 1995; Burdyga and Wary, 1997). Cytoplasmic calcium activates the contractile apparatus through the calmodulin MLCK (myosin light chain kinase) pathway (Burdyga and Wary, 1998). Several types

of Ca^{2+} currents have been characterized in the ureteral smooth muscle (Fry *et al.,* 2006). The flowing of Ca^{2+} through the activated L-type Ca^{2+} channels is considered to be the main inward current in ureteral smooth muscle (Sui and Kao, 1997a; Imaizumi *et al.,* 1989; Lang, 1989; Sui and Kao, 1997b), while T-type channels that coexist with L-type channels form the smaller component of Ca^{2+} inward current (Fry *et al.,* 2006) (Fig. 4.). A non inactivating or a slow inactivating component of Ca^{2+} generates a "window" current that determine the plateau phase of the action potential (Sui and Kao, 1997a; Imaizumi *et al.,* 1989a) as well as for providing a sustained activation of Ca^{2+} channels during prolonged depolarizing stimuli. The ureteral L-type Ca^{2+} channels are less prone to Ca^{2+} induced inactivation than in other smooth muscles (Sui and Kao, 1997a). Calcium antagonists are known to reduce ureteral contractions (Salman *et al.,* 1989). Calcium-channel blocking drugs have been used to reduce ureteral tone in patients with ureteral stones (Porpiglia *et al.,* 2002). It has been demonstrated that nifedipine (a calcium-channel blocker) and 5-methylurapidil (an α_1-receptor blocker) produced greater ureteral relaxation in vitro than diclofenac in human ureteral strips (Davenport *et al.,* 2006). Porpiglia *et al.* (2002) also demonstrated that after the use of nifedipine in patients with ureteral stones, stone-free rates were significantly greater and the time to stone passage was significantly reduced.

The sarcoplasmic reticulum contributes to excitation-contraction coupling either by the release of Ca^{2+} to initiate the contraction, or by modulation of membrane excitability (Imaizumi *et al.,* 1989; Imaizumi *et al.,* 1998; Nelson and Quayle, 1995; Carl *et al.,* 1996). Contractility of both rat and guinea pig ureter shows a major difference depending on the intracellular Ca^{2+} source (Burdyga *et al.* 1995). Using caffeine (an agonist of ryanodine receptors on the SR), or cyclopiazonic acid (a selective blocker of the SR Ca^{2+}-ATPase), as inhibitors of the sarcoplasmic reticulum will demonstrate its importance. Burdyga *et al.* (1995) found that 20 mμ caffeine at room temperature, determines a transient elevation of Ca^{2+} and contraction of the guinea pig but not of the rat. In contrast, carbachol produced a transient

elevation of Ca^{2+} and induced a rat ureteral contraction but not of the guinea pig ureter. Burdyga *et al*. (1995) also found that ryanodine and cyclopiazonic acid block the responses to caffeine in the guinea pig ureter, while only cyclopiazonic acid blocked the mobilization of Ca^{2+} in the rat ureter. Burdyga and Wray (2005) showed that disabling the (SR) with ryanodine abolished sparks and the refractory period. According to Borisova *et al.* (2007) caffeine produces a reversible inhibition of action potentials, Ca^{2+} transients and phasic contractions evoked by electrical stimulation. Whereas. It had no effect on the inward Ca^{2+} current or on the Ca^{2+} transient but increased the frequency and the amplitude of spontaneous transient outward currents in voltage clamped ureteral myocytes.

III.4.1. Ureteral action potentials and their propagation

Despite its outstanding clinical importance, the number of publications dealing with the cellular-physiological mechanisms of the ureteral smooth muscle contractility control is limited. Pressure recordings and intracellular and extracellular electrophysiological investigations of the renal pelvis and the ureter have established that a complex ‘driven’ action potential precedes every peristaltic contraction (Kuriyama and Tomita, 1970; Zawalinski *et al.*, 1975; Hannappel and Golenhofen, 1974). The ureteral smooth muscle cell membranes are polarized and maintain an electric potential in the resting, relaxed state. The cell contains a high concentration of potassium and is electrically negative. Depolarization of the membrane produces a characteristic action potential, which is conducted downward along the ureter.

We can better understand the mechanisms responsible for the characteristic long-lasting action potential by using the patch clamp studies. The electrical activity in the ureter is initiated by contractile cells with a pacemaker activity in the proximal renal pelvis (Hannappel and Golenhofen, 1974; Tsuchida *et al.*, 1981; Lang *et al.*, 1998; Lang *et al.*, 2001). This then propagates via action potentials through the ureter downwards to the bladder and causes peristaltic contractions in the smooth muscle cells (Tahara, 1990;

Smith *et al.,* 2002). The action potential of ureteral smooth muscle in normal conditions has an initial fast component consisting of repeated and gradually decaying spikes and a subsequent slow component, the so-called plateau (Fig. 9.). The initial fast single spike is due to Ca^{2+} entry when voltage gated L-type Ca^{2+} channels open, and may be truncated by the fast, but transient A-type K^+ current. The plateau phase is due to continued Ca^{2+} entry due to slowly inactivating Ca^{2+} channels (Sui and Kao, 1997a) and maintained depolarization from Ca^{2+} current, countered by outward voltage-gated and Ca^{2+} activated K^+ currents.

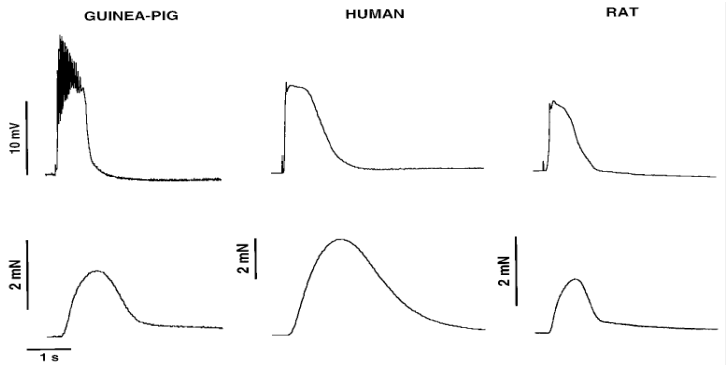

Figure 9. Action potential and accompanying phasic contraction recorded by sucrose gap from the guinea-pig, human and rat ureter. In each species the action potential is characterized by an initial fast spike followed by a long lasting plateau; in the guinea-pig ureter several oscillations superimpose onto the plateau phase of the action potential. (According to Santicioli P and Maggi CA 1998)

The human ureter action potential is characterized by its extreme length (Santicioli and Maggi, 1998), that last hundreds of milliseconds. Most of this duration is due to a pronounced plateau phase. In a study Burdyga and Wray (1999) showed that the duration of this plateau phase has an influence on the ureteral contraction via a direct influence over the Ca^{2+} transient. The action potential changes with alterations such as in stretch, metabolic and physical factors. It is influenced by the ionic concentrations of the immediate body fluids, particularly potassium, calcium, sodium, hydrogen, and other

ions (Lang *et al.*, 1998; Maggi *et al.*, 1995; Burdyga and Wray, 2002). Complexity of cellular physiological control of the action potential duration can be guessed from the work of Shabir *et al*. (2004) who showed that the rho-kinase inhibitor Y-27632 significantly reduced the duration of the action potential plateau and produced a small but significant reduction of the amplitude of the spike component.

It was reported that slow waves recorded from the smooth muscle of the rabbit renal pelvis are resistant to cholinergic, noradrenergic, and neuronal blockers to suggest a purely myogenic origin (Seki and Suzuki, 1990). The propagation of impulses occurs as a myogenic process via electrotonic spread at contact points between ureteral smooth muscle cells (Tahara, 1990). This model predicts that the interruption of action potentials at any site of the ureter will block the propagation of contraction. The signal conduction from the pacemaker site to the pyeloureteral junction is slow; multiple instances of partial or total conduction block were observed in the renal pelvis (Lammers *et al.*, 1996). It was speculated that a poor coupling between cells or stretch could be the reason of blocking the conduction within the renal pelvis (Lammers *et al.*, 1996). A partial conduction block also exists between the renal pelvis and the ureter (Constantinou and Hrynczuk, 1976), under the conditions of a normal diuresis. Not every pacemaker contraction of the renal pelvis always propagates to the ureter. It has been suggested that a urine flow dependent mechanism triggers ureteral peristalsis at the pyeloureteral junction. Stretching forces exerted on the pyeloureteral region by accumulating urine increase the coupling strength until they enable an incoming “pacemaker” wave of excitation to pass to the ureter (Constantinou. and Yamaguchi, 1981). With increasing urine flow rates, the frequency of peristaltic contractions reaches that of the pacemaker. Further increases in urine production will be accommodated by bolus volume increases, till the ureter takes the shape of an open duct (Constantinou *et al.*, 1974).

IV. Aims of the study

Improved clinical and experimental investigation techniques and the recent increase in scientific attention to smooth muscle physiology in general and ureteral physiology in particular have resulted a better understanding of the ureteral functions in health and disease. Physiologists and pharmacologists have developed objective methods by which the activity of the upper urinary tract can be monitored. The lack of noninvasive methods for investigating the intact human ureter is still one important problem. Even in vivo animal experiments are hampered by the hidden location of the upper urinary tract. In vitro techniques are of high value, but the direct applicability of their results to the in vivo situation is limited by the complicated conduction and contraction systems of the upper urinary tract.

The aim of our study 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 that the ureter was exposed by micropreparation technique developed to cause as less damage as possible, then the middle portion of the ureter were isolated from it’s surroundings by installing it in a specially designed tissue chamber that will allow the free movement of the ureter and will provide the possibility to check the effects of drugs with known influence on ureteric activity locally, also our teqnuqie provides the possibility to study the effect of systemically applied drugs through the jugular vein catheter. Using the videomicroscopic technique enabled us to follow and analyze movements of the already designated surface points, and to evaluate the phases of contraction.

V. Materials and methods

V.1. Studying ureteral peristalsis

Ureteral contractions can be studied using techniques that were mentioned in the first part of our thesis such as in vitro strips, rings and segments measuring the mechanical and electrical signals of peristalsis (Hjortswang *et al.*, 1998; Teele and Lang, 1998; Jerde *et al.*, 1999; Exintaris and Lang, 1999; Exintaris and Lang, 1999a; Lang *et al.*, 2001; Shabir *et al.*, 2004; Davenport *et al.*, 2006; Troxel *et al.*, 2006), using in vivo methods by recording intraureteral pressures (Roshani *et al.*, 1999; Moro *et al.*, 1999; Roshani *et al.*, 2002), making ultrasonographic records (Roshani *et al.* 2000), by urodynamic studies (Kinn, 1996; Lee *et al.*, 1998), and by observing the bolus movement along the ureter by videomicroscopy in rats (Lee *et al.*, 1998; Tillig and Constantinou, 1996). Nevertheless, none of these studies analyzed the complicated multidimensional movements of the ureteral wall in its natural position or the potential significance of the separate movements of the longitudinal and circular smooth muscles in the ureteral motility.

V.2. Ureteral peristalsis pattern

The ureteral peristalsis is composed of complicated multidimensional movements of the ureteral wall. Other than the changes in the diameter of the ureter due to the transverse movement there are the axial movement, axial displacement and the rotation of the ureter (Fig. 10.), all these movements combined together make it rather difficult for the available techniques to be able to study the ureteral peristalsis as a unit, because for example in the case of the rings scientists are able to study the action of the circular muscle isolated from the effect of the longitudinal muscle. For that reason it seemed advantageous to develop a new technique that will enable us to study the

ureteral peristalsis and its components not in a separate manner but as a whole.

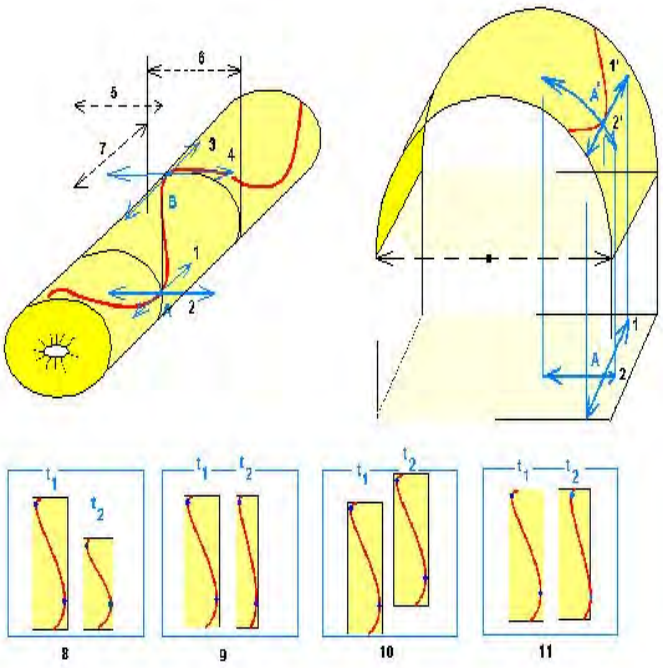

Figure 10. Geometry of ureteral contractions. 1. Axial movement of point A. 2. Transverse movement of point A. 3. Axial movement of point B. 4. Transverse movement of point B. 5. Diameter of ureter at point A 6. Diameter of ureter at point B. 7. Axial length of segment AB. 8. Axial shortening. 9. Diameter contraction. 10. Axial displacement. 11. Rotation 1' Reconstruction of the axial movement of point A on the surface 2' Reconstruction of the tangential movement of point A on the surface

V.3. Videomicroscopy

A novel technique has been developed by us to study the movements of the microsurgically exposed ureter in rats by videomicroscopy. Our studies raise the possibility that not only the amplitude and frequency of smooth muscle contractions, but also the proper synchronization of circular and longitudinal smooth muscle contractions is an important factor in forwarding the urine bolus toward the bladder.

To ensure a controlled environment in our studies the already prepared middle portion of the ureter was encased within a tissue chamber superfused with physiological salt solution (oxygenized Krebs–Ringer), the composition of which could be altered according to the specific aim of the study. Videomicroscopic pictures were carefully analyzed to reveal the motility pattern of the ureter. The movement analysis has been based on identifying certain characteristic points on the surface of the ureter determined by the vasa vasorum network (Fig. 11.). Our movement analysis is in analogy with the one that have been performed recently on the small intestinal wall by Lentle *et al.* (2007).

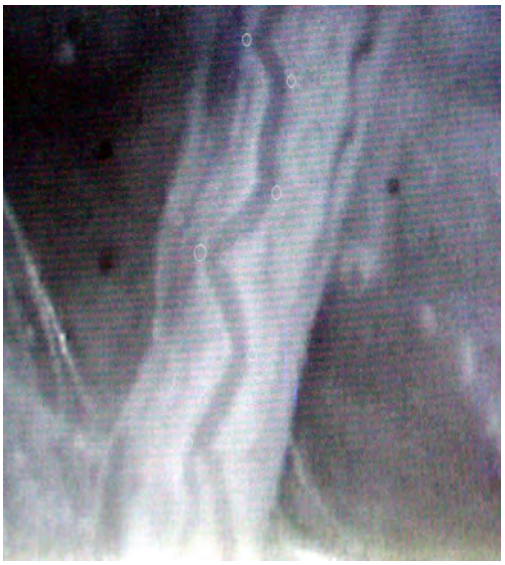

Figure 11. The movement analysis has been based on identifying certain characteristic points on the surface of the ureter determined by the vasa vasorum

V.4. Development of the tissue chambers

The microsurgical exposition of the middle portion of the ureter is one of the most important steps in our experiment. After the micro-preparation we needed to confined the middle portion of the ureter to a certain volume for two reasons, the first being to enable us to study the ureteral peristalsis in

isolation from any effect we need to isolate it from it's surrounding, the second reason was that since our experiment included the application of drugs both systematically and locally we needed to make sure that during the local application of drugs to the middle portion of the ureter there is going to be no possibility of intoxicating the subject of experiment. The only way to achieve that was to design a tissue chamber with very specific criteria that will enable us to enclose the middle portion of the ureter within it (Fig. 12.).

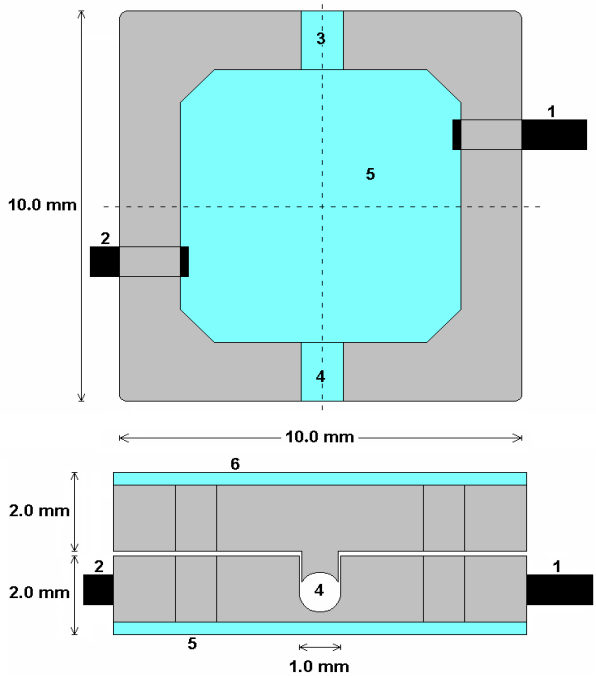

Figure 12. The tissue chamber applied to study the rat ureter in vivo pattern one. It is composed of two parts, of a base part which is positioned underneath the prepared ureter while the ureter is installed in its groove, and of an upper part that covers the ureter within the chamber. 1. Superfusion inlet. 2. Superfusion outlet. 3. Groove for entrance of ureter. 4. Groove for exit of ureter. 5. Glass bottom. 6. Glass top.

While designing the chamber we took into consideration that it should be of a small proportions to be able to fit into the limited space that we created around the ureteral middle portion through the micropreparation, to achieve that purpose the chamber diameter in our case should not exceed the one by one centimeter in diameter, as mentioned before the enclosed middle

portion of the ureter is to be studied by observing the ureteral peristalsis so the chamber has to be transparent to allow us to watch and record the contractions easily, so the material that was used in building the chamber was plastic and both the top and the bottom of the chamber was made of thin transparent plastic material. The chamber will serve as a tissue bath containing warm oxygenized Krebs–Ringer solution so during the designing process we needed to fit the chamber with an input and an output system to the solution that is appropriate to continuously and easily change the fluid, since the chamber is going to be continuously pumped with fluid, making sure that its not going to leak was one of the major things that we kept in mind while designing it especially since it was composed of two parts, a lower and an upper part, those two parts are to come together and seal the middle portion of the ureter within the tissue chamber, therefore the lower part contained four pillars one at each angel, those pillars are supposed to be inserted into four opposing holes in the lower part of the chamber and by that we insured that the chamber is not going to leak and that the two parts composing the chamber are not going to move during the experiment and while taking the videomicroscopic filming of the ureteral peristalsis. Finally, since the chamber is going to be used frequently in a limited space and have to be cleaned after every experiment we needed to make sure that it's not complicated and that it is easy to handle (Fig. 12.).

V.5. The second tissue chamber

Using the first tissue chamber started to manifest problems such as leaking the Krebs solution to the rat's abdominal cavity jeopardizing the integrity of the experiment, also the circulation system was insufficient, and finally the altitude of the chamber made it difficult for us to maneuver in the limited space that we were able to create through the micropreparation of the ureter. All these reasons caused us to create a second chamber and we tried to overcome the mistakes done in the first chamber.

The design of the second chamber took into consideration above all changing the chamber's diameter specially the altitude and the result was cutting the altitude by half after disposing the upper part of the chamber and instead of that we closed it using a thin glass top that will be installed in a built in edge in the chamber (Fig. 13.), we added a pool to the chamber to prevent the leaking of the Krebs and by that the circulation system worked better since it was able to suck the solution from the pool at the same speed of the pumping flow, the material used to build the second chamber was the same as the first one to ensure the visibility of the ureter while contracting but because of using a glass top instead of the upper plastic part of the first chamber, we were able to have a much needed clear view of the ureteral peristalsis enabling us to take a better quality video clips (Fig. 13.).

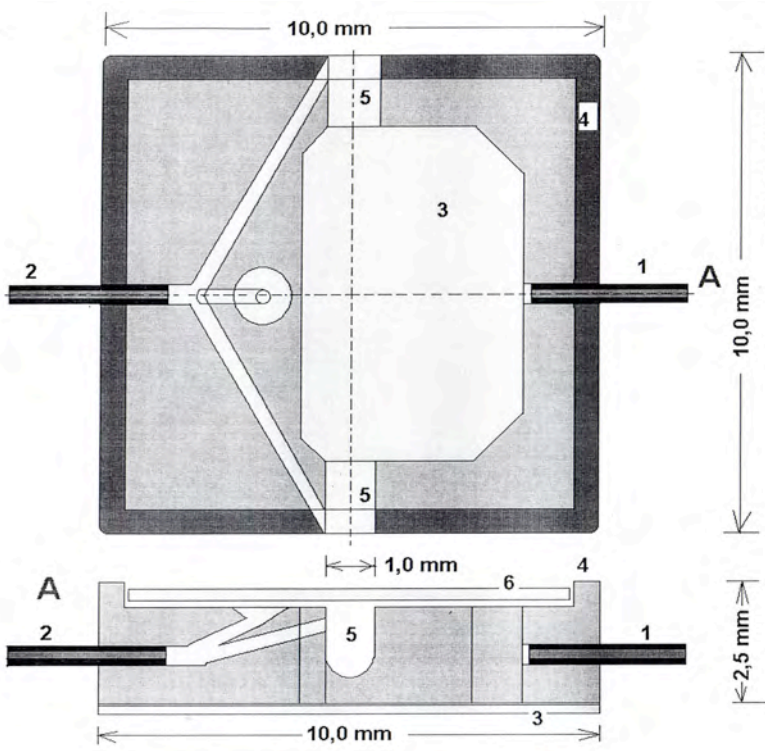

Figure 13. The second tissue chamber. 1. Superfusion inlet. 2. Superfusion outlet. 3. Glass bottom. 4. Edge. 5. Insertion for the ureter. 6. Coverslip.

V.6. Surgical exposition of the ureteral middle portion

The purpose of our study was to monitor the ureteral peristalsis and to develop a technique that will measure the contractions in a much specific way than the available techniques, the subject of our experiments were male Sprague–Dawley rats weighing 250–350 g were anesthetized with pentobarbital (Nembutal, Sanofi, 45 mg/kg body weight, given intraperitoneally) and fixed on a temperature controlled operating table. The right carotid artery were cannulated to monitor the subject of the experiment blood pressure changes through the entire duration of the micro-surgical preparation of the middle portion of the ureter, since our experiment include the drug application both systemically and locally the left jugular vein were cannulated to infuse drugs.

The abdomen was opened with a midline incision from about 10 mm over the symphysis up to about 15 mm below the xyphoid process. The intestinal mass was gently lifted and positioned on an isolating tissue and kept through the entire length of the experiment wet and warm. Since the ureter is a retroperitoneal organ a 2 cm long vertical incision was made in the left parietal peritoneum covering the hind wall of the abdominal cavity in a distance of about 1 cm from the midline at the level of the crossing of the lumbar vein. 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 because its going to be of a great value to our videomicroscopic analysis later on. The prepared middle portion is installed in the groove of the lower part of the tissue chamber (Fig. 14.).

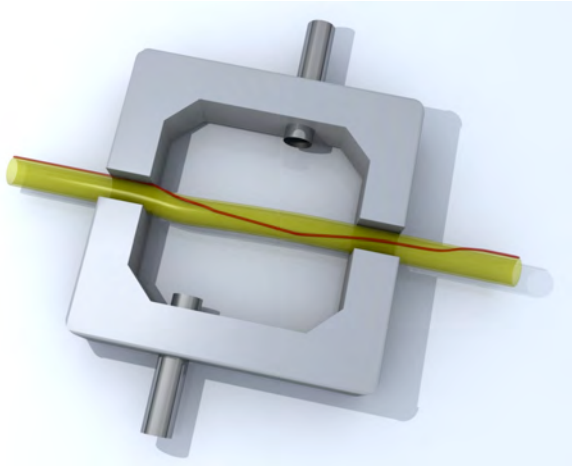

Figure 14. 3D figure of the lower part of the tissue chamber with the middle portion of the ureter installed in it. The lower part of the tissue chamber is going to be gently slide underneath the already micro-prepared middle portion of the ureter, the ureter is going to be installed in the tissue chamber groove.

The relieved ureteral section will be then encased in a tissue chamber after installing the upper part of it. The chamber with its volume around 200 cubic mm was perfused continuously at the rate of 5 ml/h with warm oxygenized Krebs–Ringer solution using a flow pump (Braun). A withdrawal pump (Harvard Apparatus) set at the same rate as the flow pump was used to remove superfluous fluid (Fig. 15.).

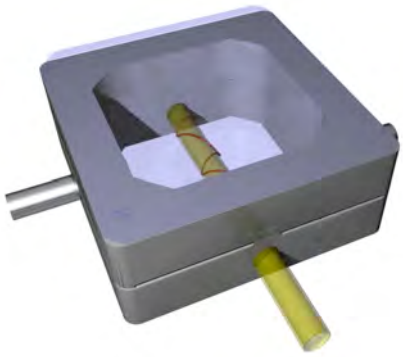

Figure 15. 3D figure of both parts of the tissue chamber (pattern one) with the middle portion of the ureter enclosed within. After the installation of the middle portion of the ureter in the groove of the lower part of the tissue chamber, the upper part of the tissue chamber is going to enclose the middle portion of the ureter within the tissue chamber.

V.7. Evaluation of ureteral motility

The ureteral peristalsis is composed of complicated multidimensional movements of the ureteral wall, it seems that the ureter is ascending upward toward the renal pelvis while the diameter of the ascending ureteral segment is increasing creating a vacuum effect that we believe is going to help into the filling of the ureteral segment with urine from the renal pelvis, after the filling of the ureteral segment it is going to descend toward the urinary bladder while the diameter of the ureteral segment just above it is going to decrease creating a circumferential ring that is going to assist in pushing the already developed ureteral bolus in the lower segment to move downward toward the bladder (Fig. 16.). The longitudinal muscles of the ureter contribute to the filling of the ureter segment and into forwarding the urine bolus toward the bladder, while the circumferential muscles of the ureter serve to enclose the urine bolus within the already expand ureteral segment and then helps into pushing the urine bolus downward to the urinary bladder (Fig. 16.).

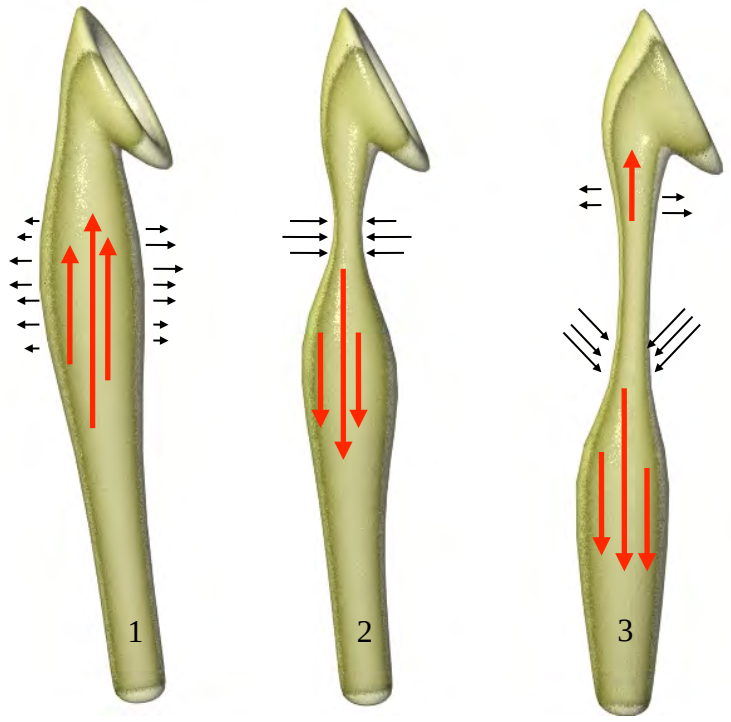

Figure 16. The thin arrows are showing the action of the circumferential muscles of the ureter, the red thick arrows are showing the action of the longitudinal muscles of the ureter. 1. The circumferential muscles are relaxing causing the ureter segment diameter to increase while the longitudinal muscles of the ureter are contracting pulling the ureter segment upward to the renal pelvis. 2. The longitudinal muscles are descending the ureter segment downward to the urinary bladder after the circumferential muscles closed the ureter segment. 3. The longitudinal muscles of the ureter are continuing moving the ureter segment downward while the circumferential muscles of the ureter are helping by pushing the ureter segment downward, simultaneously above the ureter segment that is moving downward to the bladder there is going to be a new urine bolus being created through the same cycle.

V.8. Statistical methods used to analyze periodic movements

The complexity of the ureteral peristalsis and the fact that none of the available techniques was able to study the complicated multidimensional movements of the ureter in a precise way encouraged us to study the physiology of the ureteral peristalsis and to establish a new technique that

depend on the video-microscopy to study the ureteral peristalsis. The basic concept of our technique is to take video clips of the ureter while contracting, selecting appropriate sections of it, then freezing the frames and choosing on each frame twelve cardinal points. The process is similar to the concept of the cartoon movies in which there is going to be many drawings and those drawings are to be moved in front of us to give us the sensation of a motion picture, in our case we have chosen to do the same but in the opposite way, we took the video clips freeze the frames designated the cardinal points and by that where able to follow even the finest movement of the ureteral wall (Fig. 17.).

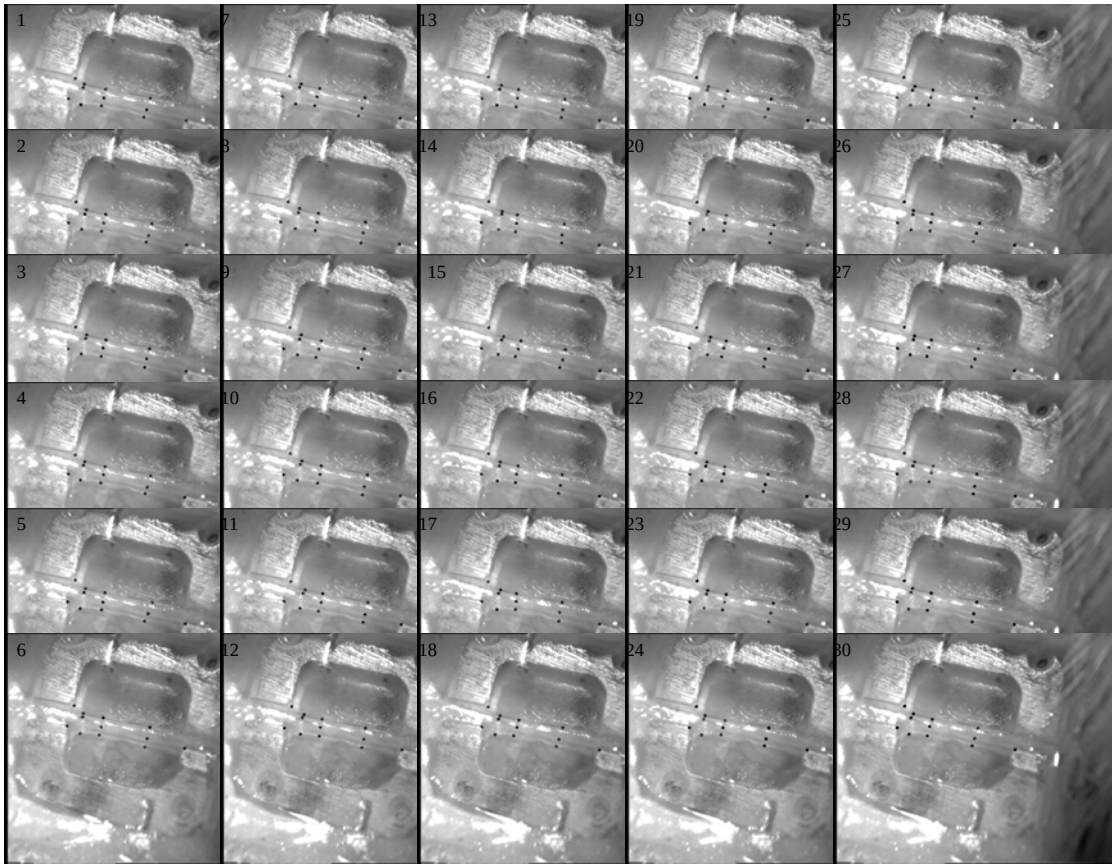

Figure 17. Thirty frames of the ureteral peristalsis after designating the cardinal points. Each six frame represent one second of the ureteral peristalsis video clip, so our figure is the total of five second video clip. The cardinal points are designated and its obvious how those cardinal points are changing their position on every frame.

We took video clips of the ureteral peristalsis for the duration of twenty seconds and converted those clips into DVD format, by using a special computer software program we were able to freeze each second of the video clips into twenty four frames, it was more than enough for us to choose six frames from each second, the result that out of twenty seconds video clip we took 120 frames, on each and every frame we designated twelve cardinal points as follows, three essential cardinal points (Av, Bv, Cv) depending on the carefully preserved vasa vasorum, and depending on the ureteral wall we designated six more cardinal points (A1, A2, B1, B2, C1, C2), the remaining three cardinal points were designated depending on the tissue chamber so we can use them as calibration points (Cal1, Cal2, Cal3) (Fig. 18a,b.).

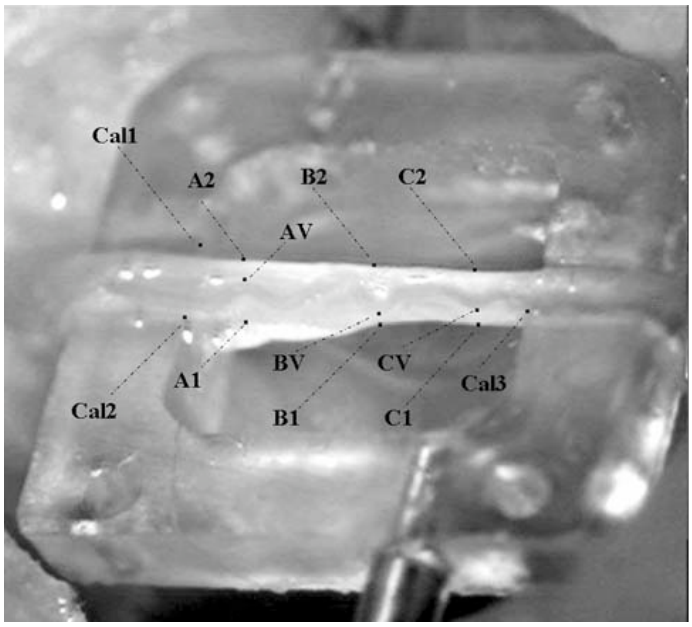

Figure 18a. Studying ureteral peristalsis using videomicroscopy: the middle portion of the ureter is encased in the tissue chamber, the twelve characteristic points whose coordinates were recorded are shown. Three fixed points on the tissue chamber Cal1, Cal2 and Cal3 were used for position calibrations. 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*. 2009a).

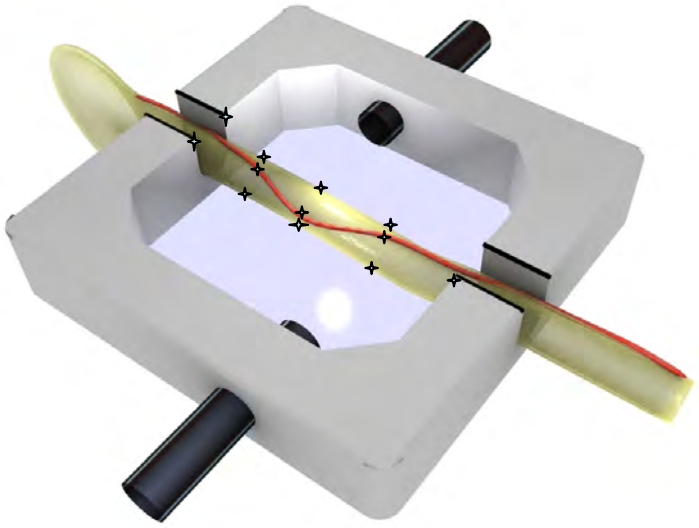

Figure 18b. A three-D figure of the ureter installed in the lower part of the tissue chamber showing the twelve cardinal points. A 3D figure showing the middle portion of the ureter installed in the tissue chamber, the three cardinal points on the frame of the chamber, the three cardinal points on the vasa vasorum already preserved through the careful micropreparation and the six cardinal points depending on the wall of the ureter.

V.9. Urine flow observation

To observe the urine flow we catheterized the left ureter orifice, a midline incision was made on the front wall of the bladder, we applied a stitch to the tip of the bladder and two other stitches to both edges of the already open bladder, we use those three stitches as retracting points to enable us to explore the bladder and to locate the ureter orifice (Fig. 19.). The left orifice is cannulated by a bent tapering plastic cannula with an outer diameter of approximately 175 micrometers at the tip (the plastic cannula is handcrafted in our lab) (Fig. 19.). Visualization of urine level movements in the cylindrical part of the plastic cannula marks volume flow as a function of time (Fig. 20.)

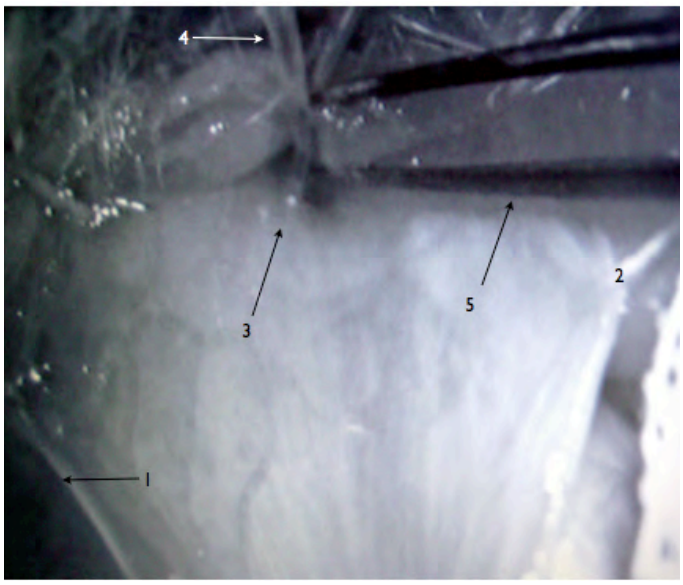

Figure 19. Rat ureter orifice catheterization 1. Bladder edge. 2. Side stitches. 3. Ureter orifice. 4. Plastic cannula. 5. Tweezer.

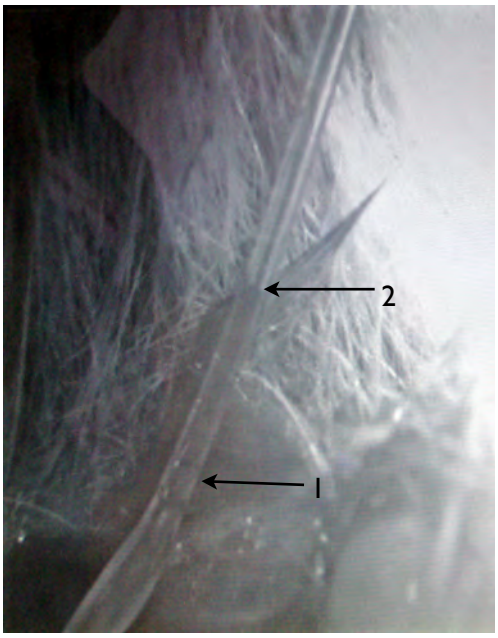

Figure 20. Movement of the urine level in the plastic cannula. 1. The catheter. 2. Rising urine level in plastic tube makes continuous recording of urine flow possible.

VI. Results

VI.1. Chamber of choice description

The tissue chamber applied to study the rat ureter in vivo. It is composed of two parts, a base part, which is positioned underneath the prepared ureter while the ureter is installed in its groove, and an upper part, that covers the ureter within the chamber. To maintain the circulation of the warm oxygenized Krebs–Ringer solution the base part of the chamber was fit with a superfusion inlet and a superfusion outlet one from each side, in the middle axle of both parts of the chamber there is a groove (Fig. 21.), the ureter is going to be installed in the groove of the base part while the groove of the upper part of the chamber is going to insure that the middle portion of the ureter is properly enclosed within the chamber without the possibility of fluid leaking or damaging the ureteral tissue. When the two parts are at there final position, they will form an opening for the entrance of the ureter and an opening for the exit of the ureter. The chamber is made of plastic with a glass bottom and a glass top to enable us to observe and record the ureteral peristalsis in a clear way (Fig. 21.).

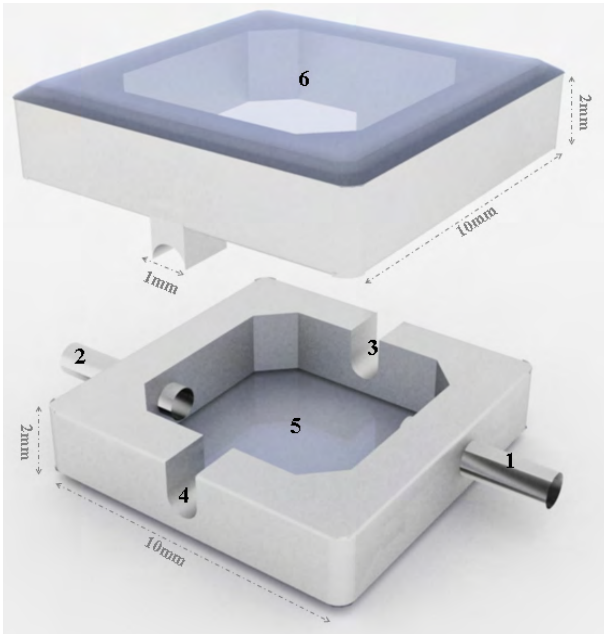

Figure 21. A three dimensional figure of the tissue chamber applied to study the rat ureter in vivo. It is composed of two parts, of a base part which is positioned underneath the prepared ureter while the ureter is installed in its groove, and of an upper part that covers the ureter within the chamber. 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*. 2009a).

VI.2. Videomicroscopis recording

Videomicroscopic pictures were recorded using a Wild M3Z preparation microscope, applying a 1.0 objective and changing the zoom settings between 10 and 40. A Philips analog video camera and videotape recorder recorded the ureteral contractions, the video clips were converted later to DVD format to enable us to use the special software program to freeze the video clips and to take frames to analyze the complicated multidimensional movement of the ureter wall. The video clips duration was set to twenty seconds, we took six frames for each second and designated the

set of twelve cardinal points on the frames depending on the ureter wall the vasa vasorum and the frame of the tissue chamber (Fig. 17.).

VI.3. Application of drugs

The aim of our study was to create a reliable accurate technique for studying the ureteral peristalsis; this technique will provide researchers with a highly accurate method to study the peristalsis and to monitor the changes that may occur due to pathological or pharmacological reasons.

In vivo and in vitro experiments were performed to explore the effects of autonomic drugs on pelvic pacemaker controlling the ureteral peristalsis Suzuki (1983) It was suggested that both noradrenaline and isoproterenol stimulated the pacemaker activity itself, while noradrenaline, elevated the renal pelvic pressure to accelerate the propagation of pacemaker activity. Consequently, the isoproterenol decreased the renal pelvic pressure to suppress the propagation consequently. Acetylcholine stimulated the pacemaker activity and its propagation transiently, but base line of renal pelvic pressure with increased contraction pressure was decreased after drug administration. Furthermore, acetylcholine sometimes developed the retrograde peristaltic contraction from ureter to pacemaker region through the pelviureteral junction. Then acetylcholine might affect directly on ureter rather than on pacemaker itself and its propagation.

Exogenous application of acetylcholine has also been shown to alter contractility in the upper urinary tract, increasing the contractility in the guinea-pig renal pelvis (Maggi and Giuliani, 1992) and equine ureter (Prieto *et al.*, 1994). In contrast, the human and guinea-pig ureter are only weakly activated by acetylcholine (Long and Nergardh, 1978; Yoshida and Kuga, 1980). In the dog ureter, acetylcholine also increases peristaltic frequency and decreases the bolus volume, although it is strange that atropine has little effect on these parameters (Morita *et al.*, 1987). Studies on the renal pelvis and ureter of the guinea-pig, rabbit and man have also demonstrated a release

of acetylcholine in response to repetitive nerve stimulation (Del Tacca, 1978). Thus, although these studies demonstrate the presence of the receptors and second messenger systems in the upper urinary tract that are normally activated by noradrenaline and acetylcholine in smooth muscle, they fail to provide evidence that a tonic release of these neurotransmitters maintains pyeloureteral motility under physiological conditions.

In our study we observed and recorded ureteral contractions and subsequently analyzed them. The application of drugs with known actions on ureteral smooth muscle was experimented in our study to observe if the motility alteration induced by them can be properly analyzed. Drugs were applied both locally by introducing them into the superfusion solution or systemically through the jugular vein cannula. Data obtained with systemic infusion of acetylcholine (5 ml/h, 0.83 mg/min) or with local infusion of acetylcholine are included in this study. The affect of the acetylcholine application was decreasing the frequency and increasing the amplitude which is typical as an effect of acetylcholine (Fig, 22a,b.). We did not try any new drug effect in our study, drugs with known effects on ureteral motility were given to check their effects on the delicate movements of different layers of the ureter. Although the application of drugs did not play an essential role in our study we think that understanding the mechanisms and paths used by drugs is essential to overall understand the ureteral motility mechanisms.

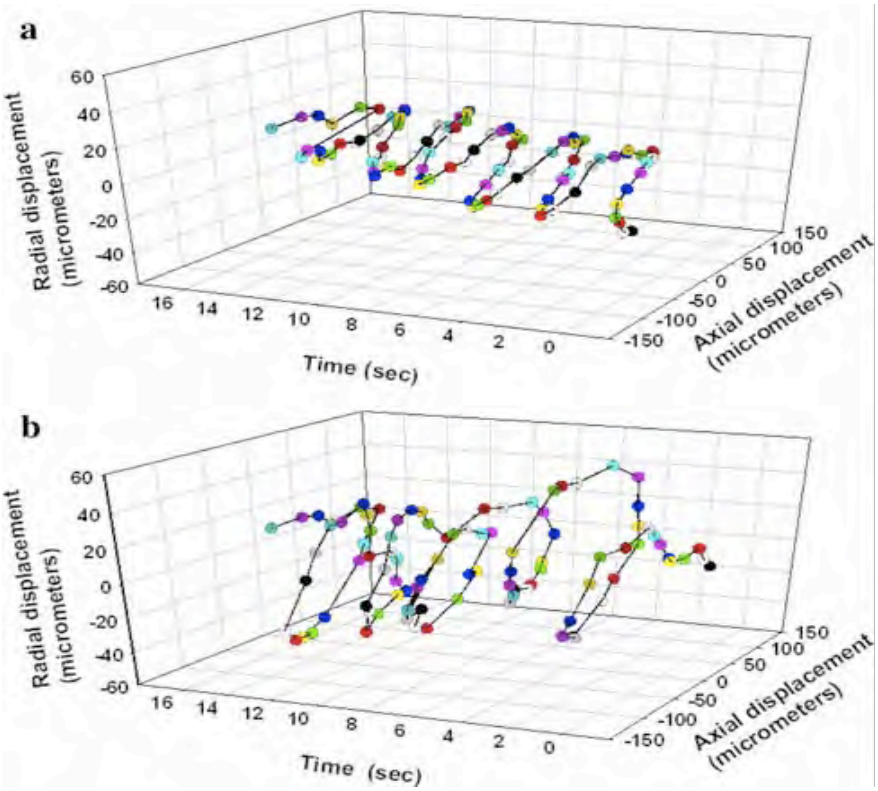

Figure 22a. Trajectory of movements of the ureter cardinal point AV shown in (Fig. 18a,b): **a** in radial and axial directions in the control state; **b** in radial and axial directions during systemic infusion of acetylcholine (0.83 _g/min). (According to Osman *et al.* 2009a).

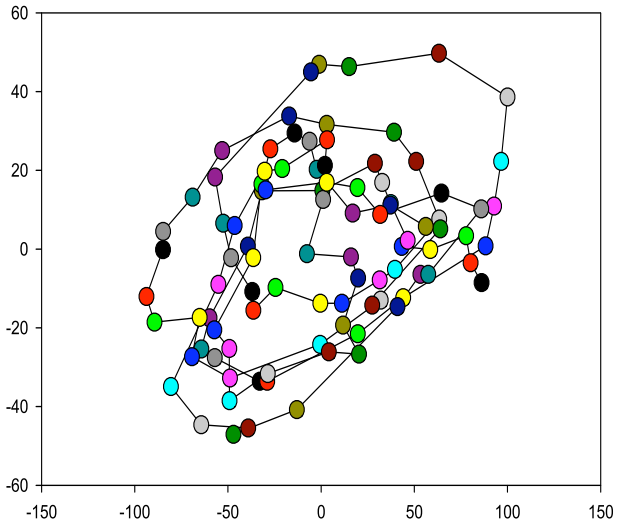

Figure 22b. Axial and radial displacements of a single point on the ureter surface projected onto the horizontal plane. Point AV shown in (Fig. 18a,b).

VI.4. Data analysis

Digitization and measurements were made offline. Digitized pictures were frozen at intervals of 166.7 ms. Characteristic points were identified and their coordinates were recorded for further computations. Calibrations were made using micrometer etalons provided by the manufacturer

(Wild, Switzerland) and known distances between characteristic points on the frame of the tissue chamber. Our scatter analysis confirmed that accuracy is limited by the size of the pixels of the digitized pictures. Space resolution limit was around 5 microm. 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. 18a,b.). Their coordinates were used to compute the movements of the ureteral wall in different directions. Movement of a characteristic point of the ureteral surface in the horizontal plane as a function of time is demonstrated using 3D Figures (Fig. 22a.). 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.

VI.5. Autocorrelation functions

The periodicity of contractions could be further analyzed by computing autocorrelation functions. Figure 23 reveals the changes in ureteral motion pattern with systemic acetylcholine application. The basal periodic time was around 1.8 s which decreased close to 1 s with the acetylcholine infusion, but

at the same time a more characteristic appearance of the 6.2 s periodic time component could be observed.

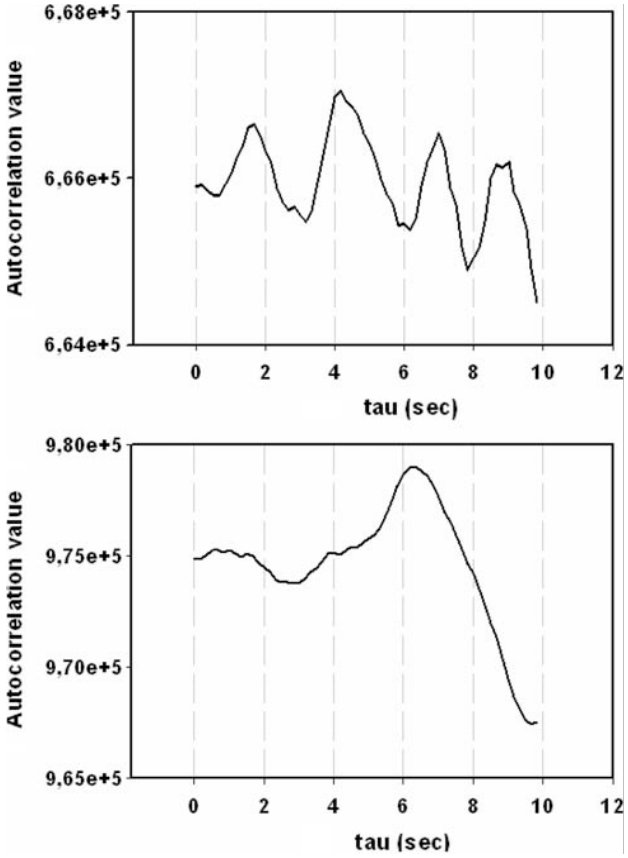

Figure 23. Autocorrelation function of diameter displacement of the cardinal point AV shown in Fig. 19a,b. The upper panel is in the control state, and the lower panel is during systemic infusion of acetylcholine (0.83 _g/min). (According to Osman *et al*. 2009a).

VI.6. Ureteral movement observation

Exposed, encased and superfused ureteral segments exhibited periodic contractile activity, the parameters of which could be altered by systemic or local application of relevant drugs. 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. 22a.) revealed a

characteristic motion pattern both in the axial and in the radial directions. The point the movement of which was analyzed in (Fig. 22a.) is located close to the ureter edge (Fig. 18a,b. point AV); still the axial movements seem to be much more extensive than the radial ones. Both frequency and amplitude altered upon systemic application of acetylcholine (Fig. 22a top and bottom records). The method provides possibility for an even more detailed analysis of ureteral movements. On (Fig. 24.) the upper record shows the radial displacement at the characteristic points, they describe the circumferential contractions at the levels of (AV and CV points in Fig. 18a,b.). The second trace shows the axial displacement of the same points; negative values mark the displacement toward the pyelon, while the positive values mark displacement toward the bladder. A negative axial displacement will be induced by a longitudinal contraction ring being above the observation site. The longitudinal contraction ring having passed below observation site will induce a positive axial displacement. The bottom trace marks the axial distance between the two characteristic points, a longitudinal shortening ring with its maximum being just in between the two observation points will induce minimum distances. These observations suggest that longitudinal smooth muscle contractions have an essential role in ureteral function.

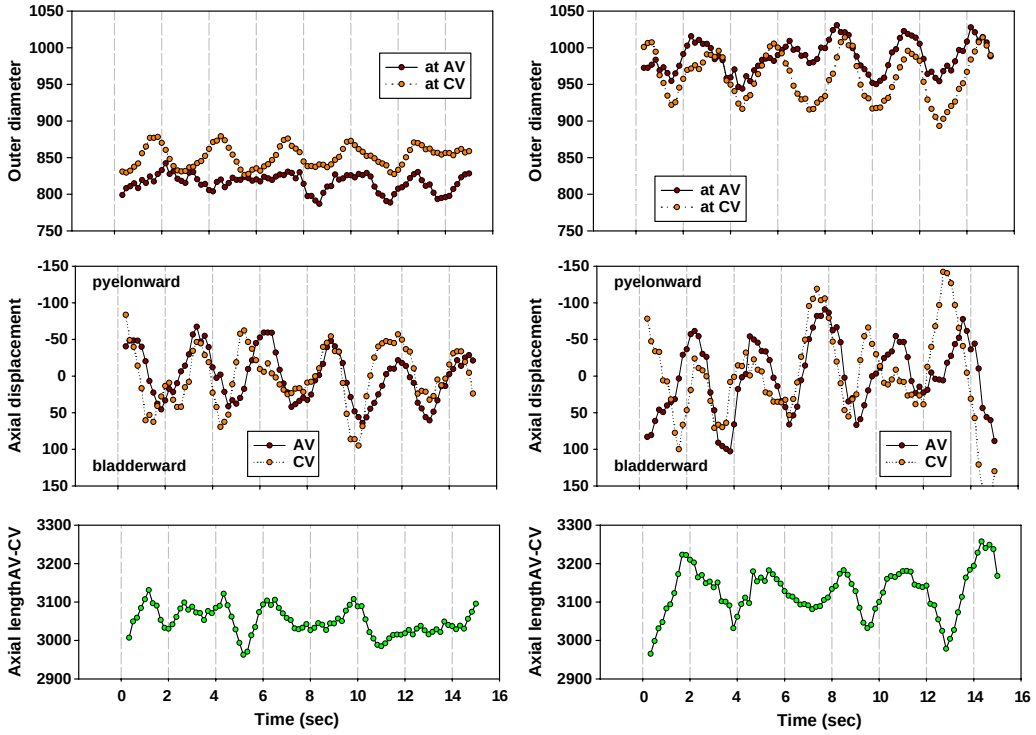

Figure 24. Upper panels of both traces show outer diameter changes at points AV and CV, Middle panels of both traces show axial displacement of points AV and CV. Lower panels of both traces show axial shortening between points AV and CV. Letters correspond to points shown on (Fig. 18a,b.). The left trace is the control state, and the right trace was recorded during systemic infusion of acetylcholine (0.83 microgram/min). (According to Osman *et al*. 2009a).

VII. Discussion

With this technique, which was developed in analogy with methods used to study intestinal movements (Lentle *et al.*, 2007), we do not follow the movements of contraction rings through an immobilized section of the animal's organ, but certain cardinal points are identified on the ureter surface and the movement of those points in a coordinate system is recorded. The longitudinal and circumferential contraction waves can be studied separately and their interaction analyzed. Classical view of urine bolus movement along the ureter attributes significance mostly to circumferential contractions. The contraction ring of this layer moves downward and pushes the urine bolus toward the bladder (Boyarsky and Labay, 1981; Lang *et al.*, 1998; Lang *et al.*, 2002). In addition, our experiments underline the significance of the longitudinal muscle contractions and shows that the longitudinal contractions play a very important role in forwarding the urine bolus downward to the urinary bladder.

VII.1. A phase analysis of ureteral movement

A detailed phase analysis of ureteral movements as measured in our experiments is shown in Fig. 25. Ureteral contractions begin with a contraction ring of the longitudinal muscle in the upper segment of the ureter. It is marked by a pyelonward displacement of the loosely tethered ureteral segments first as an effect of passive axial distention (Phases 1–2 of Fig. 25.), later as an initial active longitudinal contraction toward the maximum site of the longitudinal contraction (Phases 2–3 of Fig. 25.). A marked rise in diameter shows the lumen filling in these phases that can be considered a "diastolic period" for the observed segment. Then the circumferential contraction ring reaches the observation site, its downward movement together with the active longitudinal shortening in front of it pushes the urine

bolus downward (Phases 3–4 of Fig. 25.). At the same time active longitudinal contraction below observation point will induce a passive axial lengthening of segments above it, which helps filling the emptied segments. Then the circumferential contraction ring passes below the observation site, axial relaxation of the downward segments will release passive axial distention of the upward ones, a pyelonward movement with some diameter rise begins (Phases 4–5 of Fig. 25.).

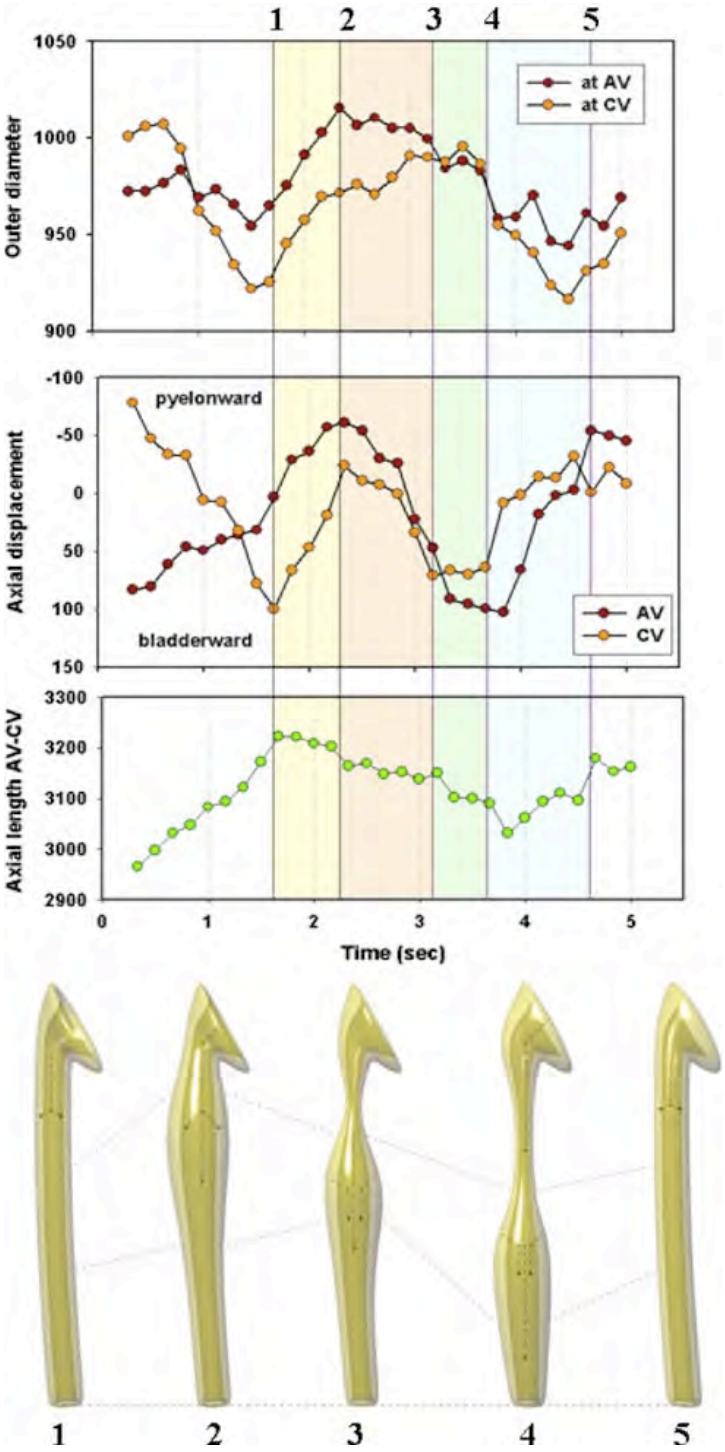

Figure 25. Suggested phases of the ureteral motion cycle: Upper panels: diameter changes at points AV and CV, axial displacement of points AV and CV, and axial shortening between points AV and CV. Letters correspond to points shown in (Fig. 18a,b.). Records were taken during systemic infusion of acetylcholine (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.* 2009a).

VII.2. Synchronization of circumferential and longitudinal contractions

All these observations underline the significance of the longitudinal smooth muscle contraction cycle and its proper synchronization with the circular smooth muscle contraction. A similar function is thought to exist in the case of intestinal longitudinal muscle (Lentle *et al.,* 2007). The situation in case of the ureter however is more complex. There is a loose axial tethering of the ureter as the position of the pyelon and of the trigone are fixed and surrounding retroperitoneal tissue restricts axial displacement more than the mesenterium in case of the small intestine. The 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.

VIII. Conclusion

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, reflux and antireflux mechanisms included. 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. Because of the axial tether of the ureter, longitudinal contractions might be more important in transporting the urine as thought earlier. For the following reasons: (1) A longitudinal contraction ring preceding the circumferential one axially distends the distal segments. (2) Initial phase of the longitudinal contraction ring promotes bolus volume rearrangement toward the passive diameter dilation. (3) Longitudinal contraction with the maximum circumferential contraction ring just behind it helps pushing the urine bolus downward. (4) Ureteral segments proximal to the longitudinal contraction ring will be passively axially stretched which also helps their filling. Thus longitudinal smooth muscle contraction helps both forming a “diastolic” phase in the ureteral motion cycle and pushing the urine bolus downward to the bladder.

IX. Summary

Ureteral motion propels urine from the renal calyces to the bladder. Despite its physiological, pathological and clinical importance our knowledge on the organization and control processes of ureteral movements is very limited. In the present Ph.D. Thesis we analyzed practically all published literature on ureteral motility, its histological, physiological, cellular and pharmacological background, and attempted to give a coherent review of our present day knowledge on the topics. For a better understanding of the mechanical events of the ureteral peristaltic cycle, the cooperation of the circular and longitudinal smooth muscle layers an in vivo technic was developed to follow the ureteral movements. With the aid of the newly developed videomicroscopic technic the synchronization of longitudinal and circular contractions could be analyzed under control conditions and under drug effects.

Analysis of the literature revealed that several histologic, cytologic and physiologic characteristics of smooth muscle in general have not yet been tested on ureteral smooth muscle. Existing data reveals that substantial differences should exist between smooth muscle of the upper and lower urinary tracts. There are many differences between ureteral smooth muscle and other smooth muscle with peristalsis, e.g. intestinal muscle. Data obtained for other types of smooth muscle should not be automatically applied for ureteral muscle. For this reason we did everything to limit the scope of our overview to observations specifically on the ureter.

A surgical technique has been developed to isolate the middle portion of the left ureter of the rat. A tissue chamber was developed that could be positioned around the ureter ensuring its continuous superfusion with saline and added drugs while through the plastic window of the chamber its movements could be videomicroscoped. Urine propagation was analyzed by recording the movement of the urine level in a micro-cannula inserted into the

left orifice. Characteristic points on the ureteral surface were identified using the pattern of the vasa vasorum on digitized, frozen videomicrosocopic pictures. Their coordinates were determined during peristaltic cycles. Steric movements were analyzed as time functions of displacements of these points. In addition, autocorrelation functions were constructed to identify periodic components. Three types of movements were separated: (i) a longitudinal displacement as a result of axial contraction outside the observation territory (ii) a longitudinal contraction/relaxation at the observation territory (iii) contraction/relaxation of the diameter. Our observations proved the ordered sequence of these movements. As a new observation, we have found that the longitudinal smooth muscle layer plays a more important role in propelling the urine bolus as it was thought earlier. Contraction of the longitudinal muscle in the mid-portion passively, axially distends the upper parts of the ureter while the circular muscle is still in the relaxed state, the result is an axial “diastolic” phase that helps filling from the calyces. The longitudinal contraction of the lower part of the ureter, followed by the circular contraction ring helps inject urine into the bladder.

The longitudinal smooth muscle layer and its coordination with the circular one plays an essential role in ureteral function. Any attempt to analyze drug effects or upper urinary tract pathology should consider that fact.

Összefoglalás

A vizeletet az ureter kontrakciói továbbítják a vesemedencéből a hólyagba. Élettani, patológiai és klinikai fontossága ellenére ma még csak kevéssé ismeretes, hogyan történik az ureter mozgásainak a szabályozása. Jelen PhD dolgozatban az ureter motilitásáról, az ureter motilitásának szövettani, élettani, sejtélettani és farmakológiai hátteréről mindezideig publikált csaknem valamennyi szakirodalmi munkát áttekintve kíséreltük meg, hogy a témakörről koherens összefoglaló képet adjunk. Az ureter perisztaltikus ciklusának, a körkörös és a hosszanti izomréteg együttes funkciójának pontosabb megértése érdekében egy új in vivo állatkisérletes technikát fejlesztettünk ki az uretermozgások tanulmányozására. Az újonnan kifejlesztett videomikroszkópos technika segítségével a hosszanti és körkörös rétegek mozgásának szinkronizálását vizsgáltuk kontroll körülmények között, valamint gyógyszerek hatását követően.

Az irodalom áttekintése meggyőzött bennünket arról, hogy számos, a simaizmot általában jellemző szövettani, sejttani és élettani sajátosság konkrét tanulmányozására ureter simaizom esetében még nem került sor. A már rendelkezésre álló anyag is arra utal, hogy a felső és az alsó húgyutak simaizomzata jelentős mértékben különbözhet egymástól. Jelentős különbség van az ureter simaizomzata és egyéb, perisztaltikus kontrakciós tevékenységet folytató izomtípusok, pl. bélizomzat között is. Ezért áttekintésünket szigorúan az ureter simaizomzatával foglalkozó cikkekre korlátoztuk.

Állatkisérletes mikrosebészeti technikát dolgoztunk ki patkány ureter középső szakaszának izolálására. Egy szöveti kamra került kifejlesztésre, mely az ureter köré helyezve biztosította annak szuperfúzióját fiziológiás sóoldattal valamint a hozzáadott gyógyszerekkel, miközben az átlátszó műanyag ablakon keresztül az uereter mozgásait videomikroszkóposan rögzíthettük. A vizelet mozgását az orificiumba illesztett plasztik mikrokanülben regisztráltuk. Digitalizált állóképeken az ureter felszín

karakterisztikus pontjait azonosítottuk a vasa vasorum mintázata alapján. Ezek koordinátáit fokozatosan követtük az ureter perisztaltikus mozgása során. Az ureter térbeli mozgását ezen karakterisztikus pontok koordinátáinak időfüggvényeként értékeltük. Kiegészítésül autokorrelációt is számítottunk a periodikus komponensek azonosítására. Az ureter mozgásának háromféle komponensét tudtuk elkülöníteni: (i) Egy pont longitudinális elmozdulása, mely az észlelés környezetétől kívül eső axiális kontrakció eredményeként jött létre. (ii) Longitudinális kontrakció/relaxáció (rövidülés/ megnyúlás) az észlelési területen. (iii) Az átmérő kontrakciója/relaxációja. Vizsgálataink igazolták, hogy ezen események rendezett sorrendben állnak elő. Új észlelésként megfigyeltük, hogy a longitudinális izomrétegnek jelentősebb a szerepe a vizelet bólus továbbításában, mint azt korábban feltételezték. A hosszanti izomzatnak a középső szakaszon való kontrakciója passzív axiális megnyúlást okoz a fölső szakaszon a körkörös izomzat relaxált állapota mellett. Ennek eredménye egy határozott axiális „diasztolés” fázis lesz, mely elősegíti a vesemedence felől történő telődést. Az ureter alsó szakaszn a hoszzanti izomzat kontrakciója, melyet az axiális kontrakciós gyűrű követ elősegíti a vizeletnek a hólyagba való injektálását.

Ezek alapján a longitudinális simaizomzat kontrakciója, ennek összerendezettsége a cirkuláris izomzat kontrakciójával lényeges szerpet tölt be az ureter funkciójában. Az ureterre ható gyógyszerek hatásának és az uretert érintő patológiai folyamatoknak az elemzésekor e tényre figyelemmel kell lenni.

X. Bibliography

X.1. General bibliography

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X.2. List of own publications

1. **Fares Osman**, György L Nádasy, Emil Monos, Péter Nyírády, Imre Romics: (2009) A novel videomicroscopic technique for studying rat ureteral peristalsis. World J Urol 27:265-270,2009. DOI: 10.1007/s00345-008-0340-6.

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2. **Fares Osman**, Imre Romics, Péter Nyírády, Emil Monos, György L Nádasy: (2009) Ureteral motility. Acta Physiologica Hungarica, Volume 96 (4), pp. 407–426 (2009) DOI: 10.1556/APhysiol.96.2009.4.2.

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X.3. Abstracts related to the Thesis

1. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Szeged, Hungary, 7-9 Június 2006). A new videomicroscopic method to study in vivo ureter movements of anesthetized rats. A Magyar Élettani Társaság (MÉT) LXX. Program. Előadások és poszterek összefoglalói P55

2. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Siófok, Hungary, 2-4 November 2006). A new videomicroscopic method to study the ureteral movements in vivo in anesthetized rats. Poster delivered at the conference of the Hungarian Urological Society.

3. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Tenerife, Spain, 10-13 December 2006). A new videomicroscopic method to study the ureteral peristaltic contractions in vivo in anesthetized rats. Oral and Poster presentation delivered at the 5th European Urological Winter Escape Meeting. (An EU-ACME accredited conference)

4. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Zagreb, Croatia, 26-27 October 2007). Measurement and analysis of ureteral perystaltic movements in anesthetized rats using a novel videomicroscopic technique. EAU 7th Central European meeting. (An EU-ACME accredited conference)

5. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Budapest, Hungary, 22-23 February 2008). Measurement and analysis of ureteral peristalitic movemens in anesthetized rats using a novel videomicroscopic technique. 19. Füvészkerti Urológus Napok – Urofarsang.

6. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Budapest, Hungary, 10-11 April 2008). A novel videomicroscopic technique for studying rat ureteral peristalsis in vivo. Semmelweis Egyetem PHD Tudományos Napok.

7. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Debrecen, Hungary, 4-6 June 2008). A novel method for measurement and analysis of ureteral peristalsis in anesthetized rats. A Magyar Kisérletes és Klinikai Farmakológiai Társaság és a Magyar Élettani Társaság LXXII. Program. Előadás és poszter összefoglalók. P. 256.

8. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Warsaw, Poland, 21-24 May 2008). A novel videomicroscopic method for studying ureteral peristalsis in vivo in anesthetized rats. The 43. Congress of the European Society for Surgical Research,

9. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Warsaw, Poland, 24-25 October 2008). A novel method to measure and analyze ureteral peristalsis in anesthetized rats. EAU 8th Central European Meeting. (An EU-ACME accreddited conference)

10. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Lublin, Poland, 11-13 September 2008). Measuring and analyzing rat ureteral peristalsis using a novel technique. 24th Congress of Polish Physiological Society.

11. **Osman F**, Nádasy GL, Monos E, Nyírády P, Romics I: (Budapest, Hungary 19-21 November 2009). Physiology of the ureter. XVIII. International Semmelweis Symposium, New trends, innovations & technology in urology.

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