Dynamics and modulation of ureteric peristalsis
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CHAPTER 8

PHARMACOLOGICAL MODULATION OF URETERIC PERISTALSIS IN A CHRONICALLY INSTRUMENTED CONSCIOUS PIG MODEL. I: EFFECT OF CHOLINERGIC STIMULATION AND INHIBITION

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ABSTRACT

OBJECTIVES: To evaluate in vivo the role of muscarinic receptors on ureteric peristaltic frequency and force of contraction in a large animal model by pharmacological manipulation.

METHODS: Twelve female pigs (72±4 kg) were chronically instrumented using an electronic pressure-monitoring catheter in the right ureter. Furthermore, nephrostomy, arterial, venous and cystostomy catheters were placed. Ureteric peristalsis was repeatedly recorded prior and subsequent to the administration of atropine and carbachol.

RESULTS: Systemic and local effects of both agents were observed. We recorded an increase of ureteric peristaltic frequency (2.0±0.3 min⁻¹, control: 1.6±0.6 min⁻¹, P<0.05) and force of contraction (50.1±1.4 cm H₂O, control 45.3±1.7 cm H₂O, P<0.05) during renal pelvis perfusion with 0.25 ml/min saline. Administration of atropine or carbachol modulated neither the force of contraction nor the frequency of ureteric peristalsis in vivo (P>0.05).

CONCLUSION: Smooth muscle motor activity at the mid and distal ureter is not modulated by muscarinic receptors. Peristaltic frequency is directly related to the pyelocalyceal load during a rate of diuresis not exceeding the "animal's own normal diuresis" plus 0.25 ml/min. Ureteric force of contraction increases only in the mid-ureter with increased diuresis.
INTRODUCTION
During normal rate of diuresis, the ureter transports urine from the kidney to the bladder by peristalsis. Myogenic and neurogenic theories were propounded to explain ureteric peristalsis. However, denervation of the ureter, kidney transplantation and even reversed ureteric auto-transplantation do not abolish peristalsis\textsuperscript{1-2}. Therefore, only the myogenic theory of ureteric peristalsis can explain why peristalsis is possible in an isolated ureteric segment \textit{in vitro} and \textit{vivo}\textsuperscript{3-4}. The development of renal colics in patients with urolithiasis proves the existence of sensory innervation of the ureter, but much less is known about neurogenic modulation of ureteric peristalsis. Nevertheless, it is well established that the mammalian ureter is extensively innervated by unmyelinated axons from different levels of the spinal cord\textsuperscript{5-8}. Using immuno-histochemistry and radio-immunoassay, considerable information has become available about the expression of receptors for neurotransmitters that presumably control neuromuscular physiology of the ureter\textsuperscript{5,7}.

We have reported earlier on the morphology and functional properties of the human and porcine ureter\textsuperscript{9-12}. A chronically instrumented animal model to investigate the physiologic relevance of the histologically demonstrated receptors for neuromuscular transmission is missing in the literature. In this article, we evaluate the effect of inhibition and stimulation of muscarinic receptors on ureteric peristalsis in a chronically instrumented conscious pig model.

The density of cholinergic nerve fibers in the ureter increases going from the renal pelvis to the bladder, the ureterovesical junction being the most densely innervated region\textsuperscript{13-14}. Acetylcholine increases the tonic and phasic contractile activity of different segments of the ureter \textit{in vitro}\textsuperscript{14-17} and also increases the peristaltic frequency\textsuperscript{18}. After nerve stimulation, acetylcholine is released from isolated renal pelvis and ureter\textsuperscript{19}. Our hypothesis therefore was that stimulation and inhibition of the muscarinic receptors \textit{in vivo} would increase and decrease, respectively, the frequency and contraction force of ureteric peristalsis.
MATERIALS AND METHODS

Experimental animals and the pre-operative procedures

Twelve female land pigs (72 ± 4 kg) were studied. Pigs were individually acclimatized and socialized during 2 weeks in the laboratory environment in a pen (2x3 meter) next to the measuring station. All experiments were monitored and registered via this station. This hall contained twenty pens and had a circadian day and night rhythm. Pigs were fed a mineral-poor laboratory pig diet. They drank and could move freely in the pen during experiments. As pre-medication ketamine (1 mg/kg) and Stresnyle® (3 mg/kg intramuscular, Janssen Farmaceutical- Holland) were used. The number of animals needed for the study was estimated using the Sigmastat® computer program based on a power of 90%. The sample size estimation revealed that nine pigs was the minimal requirement. All experiments were performed under protocols approved by the local Committee on Animal Research.

Experimental procedure-operation

Intravenous anesthesia was induced using Thiopental® (5mg/kg, Nesdonal Rhône Meurieux, France) and atropine (0.1mg/kg). Pigs were intubated and ventilated. Booster doses of Sufentanyl® (0.04 mg/kg, Janssen Farmaceutical, Holland), Midazolam (0.6 mg/kg) and Pancuronium® (0.1 mg/kg, Pavulon/Organon) were administered. Anesthesia was maintained using Sufentanyl® (0.1 ml/kg/h), Midazolam (0.13 ml/kg/h) and Pancuronium® (0.06 ml/kg/h). Animals were hydrated with 0.9% saline (approx. 10 ml/kg/h). ECG monitoring was undertaken. Peri-operative antibiotic cover was administered using 0.067 mg penicillin/kg body weight. An 8F ureteric balloon catheter endoscopically was manipulated into the distal right ureter and the balloon was lightly inflated. Under fluoroscopic control, iodine contrast was injected through the core channel of this balloon catheter to visualize the pyelocalyceal system. Under ultrasound guidance, fine needle puncture of the pyelocalyceal system was undertaken and a guide wire was positioned under X-ray control. Subsequently, the puncture trajec t was dilated telescopically. An electronic 6F pressure-monitoring catheter with twin measuring points20 was tunneled subcutaneously from the cervical area and positioned antgradely under fluoroscopic control into the right ureter. The distal and proximal measuring points lay respectively in the juxavesical portion of the ureter and 6.5 cm more proximally in the mid ureter. A pigtail nephrostomy catheter was anchore in situ. A tunneled vesicostomy catheter to measure bladder pressure was also anchored in situ via a mini-laparotomy. Separate
arterial and venous catheters were tunneled and inserted into the carotid artery and jugular vein. All tunneled catheters were protected with a Kevlar® jacket (Figure 1B).

Figure 1: The experimental set up as described in the Methods section. Measuring cables from the cervical area are protected by a Kevlar jacket (panel A) and are connected to the transducer hanging one meter above the pig. The transducer is connected to the computer in the measuring station next to the animal’s pen by a glass fiber cable. The animal can move freely during the experiment. Panel B illustrates schematically the position of different catheters and electrodes.
After the procedure, animals were allowed to recover for 9 days. During this postoperative recovery period, control registration of the ureteric peristalsis was performed each day to assess the recovery of the ureteric peristalsis after surgery of renal pelvis.

**Pressure monitoring catheter and hardware**
A special F6 measuring catheter (Gaeltec® Scotland) with a twin pressure crystal transducer was used to record peristalsis\(^{20}\). Distance between the pressure measuring points was 6.5 cm. Two electrodes were localized adjacent to each pressure transducer to measure the EMG and impedance, and served as a positive control for the peristaltic waves\(^{11,20}\). The EMG was registered using a bipolar setting and filter between 0 and 100Hz. The ECG was also registered as a superimposed signal on the EMG curves. The impedance was measured between the two intraluminally placed ureteric electrodes and an electrode on the shaved, defatted skin in the homolateral lumbar region. A skin electrode was used to earth the animal. Impedance measurements were fed using an alternating current of 500 Hz and a 12 V transducer. Direct current resistance was \(<5 \text{ K}\Omega\). The measurement box hang about 1 meter above the animal and was connected to the computer using a glass fiber cable (Figure 1). Data were registered using LabVIEW (National Instrument®, USA) software running on a Windows NT4® operating system. The hard- and software was specially developed in collaboration with the Department of Biophysics at the University of Amsterdam and BIOSEMI® (The Netherlands). The catheter was zeroed before introduction at operation and was again controlled at every data registration session.

**Monitoring of haemo- and urodynamic signals**
A multichannel Hewlett Packard (HP-78342A) pressure transducer was used to register the blood pressure via the arterial catheter. The jugular venous catheter was used for drug administration. To register the hydrostatic pressure in the renal pelvis and the bladder, the nephrostomy and cystostomy catheters were used. The pressure transduction chambers were flushed and zeroed regularly at the level of the organ concerned.

**Care and maintenance of animals**
Each animal was examined daily. In all animals, urinary leakage from the nephrostomy ceased within 24 hours. Urinary sediment and culture samples were collected and were always negative. Daily physical examination
revealed no evidence of pyelonephritis. Ultrasound studies (B&K 3535) of the kidneys were undertaken before every data registration session. Only one animal revealed dilatation of the pyelocalyceal system and was excluded from the study. Nursing care of the animals was also regularly undertaken by the investigator to cultivate and develop a social bond as well as reduce animal stress to minimum during the study.

**Drug administration and data registration**

At the start of every session, a physical examination of the pig under study as well as an ultrasound examination of its upper urinary tract was performed. Furthermore, baseline urine production and, blood pressure as well as pressure in the renal pelvis and bladder during approximately 30 minutes were checked. In addition, ureteric EMG, impedance and the pressure changes due to peristalsis were registered during a period of 1000 sec and used as reference (REF-1). Diuresis during this REF-1 period was measured by emptying the bladder via the vesicostomy. The percutaneous nephrostomy catheter was then perfused with 0.25 ml/min saline at body temperature using a pump (Becton-Dickinson, England) and the above mentioned registration was repeated as a second control (REF-2) to be sure that a pyelocalyceal load was present (360 ml/24h plus endogenous diuresis). This control study was important because when due to drug administration a blood pressure drop occurs and diuresis decreases, this baseline provides guidance. Agonists and antagonists of muscarinic, beta- and alfa adrenergic and nitrergic receptors were administered intravenously according to a pre-planned rotating schedule at 3-day intervals to eliminate any drug interaction. Systemic effects of medication were registered. In the current study, carbachol (0.004 mg/kg) and atropine (0.5 mg/kg) were administered as muscarinic agonist and antagonist, respectively. These doses were chosen based on available human and veterinary literature. Every experiment lasted 6 weeks.

**Animal euthanasia**

Pigs were sedated as described. X-ray imaging of the upper tract was undertaken after perfusion (0.25 ml/min) of the nephrostomy with dilute iodine contrast as described earlier to confirm the presence of normal peristalsis 6 weeks post-operatively. The intraluminal catheter was found to have advanced approximately two cm proximally in all cases in comparison to its position on the day of introduction (due to animal growth and/or movement). Pigs were then euthenised in accordance with laboratory
guidelines (10 ml pentobarbital sodium 200 mg/ml I.V.). The urinary tracts were immediately harvested for histological analysis.

Data analysis
The amplitude (\( P_{\text{max}} \), cm H2O) of every pressure signal, its duration (sec) and frequency (min\(^{-1}\)) of the peristaltic curves were manually analyzed and recorded in a spreadsheet program. The duration of pressure signal is converted in pressure signal length (mm) by multiplying duration with propagation velocity\(^{12}\). Frequency distribution analysis of our data revealed a natural distribution that justifies the determination of the mean, standard error of the mean (SEM) and the P value using a two tailed, unpaired student t-test.

RESULTS
We used the same criteria to identify a peristaltic wave as reported earlier\(^{20}\). Registration of the curves was possible in all sessions. In two pigs, registration of only one channel was possible due to a break in the wire of the other channel. One pig destroyed the measuring catheter, so that a second operation was necessary to replace the damaged catheter.

Quantitative description of the registered peristalsis
Quantitative analysis of the acquired data from the REF-1, REF-2, atropine, and carbachol sessions was undertaken using the Excel 2000 (Microsoft\(^{8}\)) spreadsheet program.

Systemic effects: Atropine and carbachol caused a significant increase (180 ± 6 min\(^{-1}\)) and decrease (56 ± 3 min\(^{-1}\)), respectively, in heart rate (control: 83 ± 4 min\(^{-1}\), P<0.05). Blood pressure was not affected. The average diuresis of the pigs during atropine and carbachol was 64.6 ± 0.8 ml/hour and 62.6 ± 0.7 ml/hour, respectively, and did not differ significantly from the control condition (63.9 ± 0.2 ml/hour). Atropine caused a dry cough and mydriasis, whereas carbachol initiated a massive saliva production.

Urological effects: Post-operative measurement of the ureteric peristalsis within 4 hours after the implantation of the catheter revealed presence of ureteric peristalsis with a frequency of 1.4 ± 0.7 min\(^{-1}\) and \( P_{\text{max}} \) of 39.2 ± 1.6 cm H\(_2\)O in the mid-ureter. Ureteric peristalsis vanished at day 1 and 2 after the operation. The ureteric activity reappeared gradually during the
subsequent days. Hydrostatic pressure in the renal pelvis became measurable again on the 3rd post-operative day. Its rhythmicity reappeared on the 4th post-operative day, with peaks of 7 cm H₂O and dips of 1 cm H₂O. Up to day 5, peristaltic activity in the mid-ureter were irregular in amplitude and occurred in clusters, followed by long inactive period. Single wave patterns ensued from day 7 onwards. Normal ureteric peristalsis in distal ureter was re-established a day later than in the mid-ureter.

The variation of the maximal pressure during a peristaltic wave (P_{max}) in the REF-1 and REF-2 periods, as well as the effects of atropine and carbachol is summarized in Table 1.

<table>
<thead>
<tr>
<th>Antagonist</th>
<th>Experimental Condition</th>
<th>P_{max} prox (cm H₂O)</th>
<th>P_{max} distal (cm H₂O)</th>
<th>Length of prox signal (mm)</th>
<th>Length of distal signal (mm)</th>
<th>Frequency (min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>REF-1</td>
<td>*45.3 ± 1.7</td>
<td>46.5 ± 1.7</td>
<td>26.0 ± 1.7</td>
<td>25.5 ± 1.7</td>
<td>*1.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>REF-2</td>
<td>*50.1 ± 1.4</td>
<td>48.6 ± 1.5</td>
<td>25.6 ± 1.5</td>
<td>27.6 ± 1.5</td>
<td>*2.0 ± 0.3</td>
</tr>
<tr>
<td>Carbachol</td>
<td>REF-1</td>
<td>51.4 ± 1.4</td>
<td>50.3 ± 1.5</td>
<td>28.3 ± 1.5</td>
<td>26.3 ± 1.5</td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>REF-2</td>
<td>*43.7 ± 1.5</td>
<td>53.4 ± 2.0</td>
<td>29.3 ± 1.8</td>
<td>27.1 ± 2.0</td>
<td>*1.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>Carbachol</td>
<td>49.4 ± 1.9</td>
<td>50.7 ± 2.0</td>
<td>30.1 ± 2.1</td>
<td>27.6 ± 1.9</td>
<td>1.6 ± 1.1</td>
</tr>
</tbody>
</table>

Table 1: Reference values before (RE-1) and after (REF-2) perfusion of the upper urinary tract with 0.25 ml/min saline, and after administration of atropine or carbachol. Note that P_{max} and peristaltic frequency in mid-ureter are significantly increased during perfusion of the renal pelvis with 0.25 ml/min saline (cf. REF-2 with REF-1). Atropine and carbachol failed to affect the ureteric peristalsis in any of its parameters when compared with REF-2 results. Asterisk: P <0.05. Results: Mean ± SEM

P_{max}, the length of the pressure wave and the peristaltic frequency were unaffected by either atropine or carbachol. Perfusion of 0.25ml saline through the nephrostomy caused a significant (P<0.05) increase of P_{max} and peristaltic frequency, but in the mid-ureter only. Hydrostatic pressure in the renal pelvis showed a rhythmic variation between 0 and 6.5 cm H₂O that was non-synchronous with respiration and that was also unaffected by atropine or carbachol. The two drugs did not change intravesical pressure either. Imaging studies with X-rays revealed that peristalsis was still present at 6 weeks postoperatively. The measuring catheter was displaced approximately two cm proximal in comparison with its level on the day of introduction. Histological
staining (haematoxylin & eosin) of the harvested ureter revealed only a very mild inflammatory response.

DISCUSSION

Importance of chronically instrumented animal studies vs. acute experiments

A considerable number of studies on ureteric physiology that are based on *in vitro* or acute *in vivo* experiments \(^{14-17,21-25}\) have been published. The most important criticism of these data is the influence of anesthesia and trauma on normal physiology. Results of *in vitro* studies can never fully explain the effects of a complex and dynamic multivariate regulatory circuit e.g. the effects of receptor stimulation or inhibition on motor activity in the ureter. The mammalian upper urinary tract (UUT) is also difficult to reach since it is hidden safely in the retroperitoneal area. Radioactive imaging techniques (MAG-3 renogram) as used in clinical studies of UUT motility are unsuitable for experimental study in a conscious chronically instrumented animal. Movement artifacts would render the study impossible or useless. Our experimental set up is similar to that used in humans for percutaneous nephrolithotripsy (PNL) with a double pigtail catheter left *in situ*. Our detailed control studies reveal that the UUT of our animals was not affected to any significant degree by inflammation or signs of obstructive uropathy.

Extensive searches in the Med-line database failed to reveal reports with a similar approach to our physiological study of the UUT. There were several technical problems to overcome. Successful puncture of a minimally dilated UUT of the pig is more difficult than in man because of the long thoracic cage covering the kidney. Pigs are by nature intelligent, but routing, inquisitive and destructive animals. It was therefore a great challenge to position and maintain all tunneled measuring catheters *in situ* during the weeks of experimentation. Despite all our efforts, we did lose one catheter as mentioned. Animal movement negatively affects the registration of small signals. To this end, the pigs socialization program with the investigator was of crucial importance to reduce the number of artifacts to a minimum and to assure the success of the experiments.
Discrepancy between the in vivo and in vitro studies

Cholinergic nerve fibers are present in various densities in the UUT \(^{13-14}\). They are reported to be of the muscarinic type in most species, including pigs \(^{17,23-25}\). *In vitro*, acetylcholine is reported to have an inotropic and chronotropic effect on the UUT \(^{14-18}\). Since systemic effects of cholinergic stimulation and inhibition were observed in all our animals, there can be no doubt that an effective biological dose of the drug was administered. Furthermore, our experimental set-up was calibrated to register changes in frequency and contraction force of ureteric peristalsis when the renal pelvis was perfused with 0.25 ml/min saline. Our results therefore appear to negate any functional relevance of the muscarinic receptors in the UUT of the conscious pig. The reason why cholinergic receptor manipulation fails to show any functional response is puzzling, but possibilities are that other regulatory circuits are superimposed on cholinergic receptor function or that the cholinergic receptors are involved in sensory rather than motor pathways. Although the drugs in the concentrations used were effective on the heart and glands, their local concentration in the UUT may also have been too low to effectively influence its function.

Presumed clinical relevance

Anti-cholinergic (anti-muscarinic) drugs are used in clinical urology to treat bladder motor hyper-reflexia and renal or ureteric colics. The mechanistic hypothesis underlying this therapy is that an anti-muscarinic drug would reduce hyper-contractility and spasms of the bladder and ureter. Based on our experiments, this spasmolytic effect does not exist. In agreement, no clinical evidence has also ever been reported that continuous use of anti-cholinergic medication over a long period when used for bladder hyper-reflexia resulted in a dilatation of the UUT. However, the observed co-localization of muscarinic receptors with Calcitonin Gene Related Peptide-positive nerves \(^{26-27}\) may suggest a sensory function for muscarinic receptors. Anti-cholinergic agents may therefore provide relief from pain of ureteric or renal colics via this pathway. We are nevertheless aware that species differences may play an important role in any experimental animal model.
CONCLUSION
Ureteric peristalsis persists in initial hours after the operation at pyelocalyceal system. Normal function is re-established a week after the manipulation. Recovery follows a gradual and hierarchic pattern from proximal to distal.

At low rates of diuresis, peristaltic frequency is directly related to the pyelocalyceal urine load. Although ureteric contraction force is increased in the mid-ureter when diuresis rate is increased, $P_{\text{max}}$ fails to increase in distal, juxtavesical ureter.

Ureteric smooth muscle motor activity at the mid- and distal ureter level is not modulated by muscarinic receptors in our chronically instrumented animal model. The presumed spasmolytic effect of anti-cholinergic therapy for renal colics is therefore not supported by our findings.

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