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

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ABSTRACT

Purpose: We evaluated in vivo the role of muscarinic receptors on ureteral peristaltic frequency and contraction force in a large animal model using pharmacological manipulation.

Materials and Methods: A total of 12 female pigs weighing a mean ± SEM of 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. Ureteral peristalsis was repeatedly recorded before and after the administration of atropine and carbamol.

Results: Systemic and local effects of the 2 agents were observed. Compared with controls we recorded an increase in mean ureteral peristaltic frequency (2.0 ± 0.3 versus 1.6 ± 0.6 minutes⁻¹, p < 0.05) and mean contraction force (50.1 ± 1.4 versus 45.3 ± 1.7 cm H₂O, p < 0.05) during renal pelvis perfusion with 0.25 ml per minute saline. Administration of atropine or carbamol modulated neither the force of contraction nor the frequency of ureteral peristalsis in vivo (p > 0.05).

Conclusions: Smooth muscle motor activity at the mid and distal ureter is not modulated by muscarinic receptors. Peristaltic frequency is directly related to the pyelocaliceal load during a rate of diuresis not exceeding animal normal diuresis plus 0.25 ml per minute. Ureteral contraction force increases only in the mid ureter with increased diuresis.

KEY WORDS: ureter; swine; peristalsis; diuresis; muscle, smooth

During the normal rate of diuresis the ureter transports urine from kidney to bladder by peristalsis. Myogenic and neurogenic theories have been propounded to explain ureteral peristalsis. However, denervation of the ureter, kidney transplantation and even reversed ureteral autotransplantation do not abolish peristalsis. Therefore, only the myogenic theory of ureteral peristalsis can explain why peristalsis is possible in an isolated ureteral segment in vitro and in vivo. The development of renal colic in patients with urolithiasis proves the existence of sensory innervation of the ureter but much less is known about neurogenic modulation of ureteral peristalsis. Nevertheless, it is well established that the mammalian ureter is extensively innervated by unmyelinated axons from different levels of the spinal cord. Using immunohistochemistry and radioimmunoassay considerable information has become available about the expression of receptors for neurotransmitters that presumably control neuromuscular physiology of the ureter.

We have previously reported the morphology and functional properties of the human and porcine ureter. A chronically instrumented animal model to investigate the physiological relevance of histologically demonstrated receptors for neuromuscular transmission is missing in the literature. In this study we evaluated the effect of inhibition and stimulation of muscarinic receptors on ureteral peristalsis in a chronically instrumented, conscious pig model.

The density of cholinergic nerve fibers in the ureter increases from renal pelvis to bladder with the ureterovesical junction the most densely innervated region. Acetylcholine increases tonic and phasic contractile activity of different segments of the ureter in vitro and also increases peristaltic frequency. After nerve stimulation acetylcholine is released from isolated renal pelvis and ureter. Therefore, our hypothesis was that stimulation and inhibition of muscarinic receptors in vivo would increase and decrease, respectively, the frequency and contraction force of ureteral peristalsis.

MATERIAL AND METHODS

Experimental animals and preoperative procedures. A total of 12 female land pigs weighing a mean ± SEM of 72 ± 4 kg were studied. Pigs were individually acclimatized and socialized during 2 weeks in the laboratory environment in a 2 × 3 m pen next to the measuring station. All experiments were monitored and documented via this station. This hall contained 20 pens and had a circadian day and night rhythm. The pigs were fed a mineral poor laboratory pig diet. They drank and could move freely in the pen during experiments. As premedication 1 mg/kg ketamine and 3 mg/kg Stresnily (Janssen Farmaceutical, Tilburg, The Netherlands) were used. The number of animals needed for the study was estimated using the Sigmastat (SPSS Science, Chicago, Illinois) computer program based on a power of 90%. Sample size estimation revealed that 9 pigs were the minimal requirement. All experiments were performed under protocols approved by the local committee on animal research.

Experimental operation. Intravenous anesthesia was induced using 5 mg/kg thiopental (Nesdonal Rhone Mureieux, Lyon, France) and 0.1 mg/kg atropine. The pigs were intu-
bated and ventilated. Booster doses of 0.04 mg/kg Sufentanyl (Janssen Farmaceutical) 0.6 mg/kg midazolam and 0.1 mg/kg pancuronium (Pavulon/Organon, Oss, The Netherlands) were administered. Anesthesia was maintained using 0.1 ml/kg per hour Sufentanyl, 0.13 ml/kg per hour midazolam and 0.06 ml/kg per hour pancuronium. The animals were hydrated with 0.9% saline (approximately 10 ml/kg per hour). Electrocardiogram monitoring was done. Perioperatively antibiotic coverage was administered using 0.067 mg penicillin per kg body weight. An 8Fr ureteral balloon catheter was manipulated endoscopically into the distal right ureter and the balloon was lightly inflated. Under fluoroscopic control iodine contrast medium was injected through the core channel of this balloon catheter to visualize the pyelocaliceal system. Under ultrasound guidance fine needle puncture of the pyelocaliceal system was performed and a guide wire was positioned under x-ray guidance. Subsequently the puncture tract was dilated telescopically. An electronic 6Fr pressure monitoring catheter with twin measuring points was tunneled subcutaneously from the cervical area and positioned antegrade under fluoroscopic guidance into the right ureter. The distal and proximal measuring points lay respectively in the vesicoperitoneal region of the ureter and 6.5 cm more proximal in the mid ureter. A pigtail nephrostomy catheter was anchored in situ. A tunneled vesicostomy catheter to measure bladder pressure was also anchored in situ via minilaparotomy. 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 (van Baal, Utrecht, The Netherlands, see figure). After the procedure the animals were allowed to recover for 9 days. During this postoperative recovery period followup documentation of ureteral peristalsis was performed on day 6 to assess the recovery of ureteral peristalsis after surgery on the renal pelvis.

**Pressure monitoring catheter and hardware.** A special 6Fr Gaeltec (Dunvegan, Isle of Skye, Scotland) with a twin pressure crystal transducer was used to record peristalsis. The distance between the pressure measuring points was 6.5 cm. Two electrodes were localized adjacent to each pressure transducer to measure EMG and impedance, and serve as a positive control for the peristaltic waves. EMG was recorded using a bipolar setting and filter between 0 and 100 Hz. Electrocardiogram was also recorded as a superimposed signal on the EMG curves. Impedance was measured between the 2 intraluminally placed ureteral electrodes and an electrode on the defatted skin in the homolateral lumbar region. A skin electrode was used to earth the animal. Imedication measurements were fed using an alternating current of 500 Hz and a 12 V transducer. Direct current resistance was less than 5 KΩ. The measurement box was about 1 m above the animal and connected to the computer using a glass fiber cable. Data were recorded using LabVIEW (National Instruments Corp., Austin, Texas) software on a Windows NT4 (Microsoft, Redmond, Oregon) operating system. The catheter was set to zero before introduction at operation and again controlled at each data recording session. The criteria to identify a peristaltic wave were those reported earlier. Documentation of the curves was possible at all sessions. In 2 pigs recording only 1 channel was possible due to a broken wire in the other channel. Another pig destroyed the measuring catheter, so that a second operation was necessary to replace the damaged catheter.

**Monitoring of hemodynamic and urodynamic signals.** A multichannel HP-78342A (Hewlett-Puckard, Andover, Massachusetts) pressure transducer was used to record blood pressure via the arterial catheter. The jugular venous catheter was used for drug administration. To document hydrodynamics pressure in the renal pelvis and cystostomy catheters were used. The pressure transduction chambers were flushed and set to zero regularly at the level of the organ concerned.

**Animal care and maintenance.** Each animal was examined daily. In all animals urinary leakage from the nephrostomy tube ceased within 24 hours. Urinary sediment and culture samples were collected and always negative. Daily physical examination revealed no evidence of pyelonephritis. Ultrasound of the kidneys using a 3535 (B-K, 3535, B-K Medical, Herlev, Denmark) device was done before each data recording session. Only 1 animal had dilatation of the pyelocaliceal system and it was excluded from study. Nursing care of the animals was also regularly done by the investigator to cultivate and develop a social bond as well as decrease animal stress to a minimum during the study.

**Drug administration and data documentation.** At the start of each session physical examination of the pig under study as well as ultrasound 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 assessed. In addition, ureteral EMG, impedance and pressure changes due to peristalsis were recorded during a period of 1,000 seconds and used as reference (REF-1). Diuresis during this REF-1 period was measured by emptying the bladder via the vesicostomy. These drugs were also administered at 3-day intervals to eliminate any drug interaction. Systemic effects of medication were noted. In the current study 0.004 mg/kg carbachol and 0.5 mg/kg atropine were administered as muscarinic agonist and antagonist, respectively. These doses were chosen based on available human and veterinary literature. Each experiment lasted 6 weeks.

**Terminal assessment of ureteral function.** The pigs were sedated as described. X-ray imaging of the upper tract was done after perfusion (0.25 ml per minute) of the nephrostomy with dilute iodine contrast medium as described to confirm the presence of normal peristalsis 6 weeks postoperatively. Peristaltic activity was similar to that observed during the
initial operation but the intraluminal catheter was seen to have advanced approximately 2 cm proximal in all cases compared with its position on the day of introduction (due to animal growth and/or movement). The pigs were then sacrificed in accordance with laboratory guidelines (10 ml pentobarbital sodium 200 mg/ml intravenously). The urinary tracts were immediately harvested for histological analysis.

Data analysis. The amplitude (Pmax) in cm H2O of each pressure signal, its duration (in seconds and frequency in minutes−1) of the peristaltic curves were manually analyzed and recorded in a spreadsheet program. The duration of the pressure signal was converted in pressure signal length in mm by multiplying duration by propagation velocity.12 Frequency distribution analysis of our data revealed a natural distribution that justified determination of the mean ± SEM and p value using the 2-tailed unpaired Student t test.

Quantitative description of documented peristalsis. Quantitative analysis of the acquired data from the REF-1, REF-2, atropine and carbachol sessions was done using the Excel 2000 spreadsheet program.

RESULTS

Systemic effects. Atropine and carbachol caused a significant increase (180 ± 6 minutes−1) and decrease (56 ± 3 minutes−1), respectively, in heart rate (control 83 ± 4 minutes−1, p < 0.05). Blood pressure was not affected. Average diuresis of the pigs during atropine and carbachol was 64.6 ± 0.8 and 62.6 ± 0.7 ml per hour, respectively, and it did not differ significantly from the control condition (63.9 ± 0.2 ml per hour). Atropine caused a dry cough and mydriasis, whereas carbachol initiated massive saliva production.

Urological effects. Postoperative measurement of ureteral peristalsis within 4 hours after catheter implantation revealed ureteral peristalsis at a frequency of 1.4 ± 0.7 minutes−1 and a Pmax of 39.2 ± 1.6 cm H2O in the mid ureter. Ureteral peristalsis vanished on days 1 and 2 after the operation. Urine production was similar during these 2 days (62.8 ± 0.8 ml per hour) compared with the other postoperative periods (33.4 ± 0.6 ml per hour). We did not perform fluoroscopic imaging to avoid a second anesthesia but ultrasonography did not reveal detectable dilatation of the renal pelvis. Ureteral activity reappeared gradually during the subsequent days. Up to day 5 peristaltic activity in the mid ureter was irregular in amplitude and occurred in clusters, followed by a long inactive period. Single wave patterns ensued from day 7 and thereafter. Normal ureteral peristalsis in the distal ureter was reestablished a day later than in the mid ureter. Hydrostatic pressure in the renal pelvis became measurable again on postoperative day 3. Its rhythmicity reappeared on postoperative day 4 with peaks of 7 cm H2O and dips of 1 cm H2O. The rhythmicity in hydrostatic pressure in the renal pelvis did not correlate with ureteral peristaltic frequency.

The table lists variations in maximal pressure during a peristaltic wave or Pmax in the REF-1 and REF-2 periods as well as the effects of atropine and carbachol. Average baseline pressure or REF-1 did not fluctuate significantly during experiments in individual pigs but differences were seen between pigs. Pmax, the length of the pressure wave and peristaltic frequency were unaffected by atropine or carbachol. Perfusion of 0.25 ml saline through the nephrostomy caused a significant increase of Pmax and peristaltic frequency but in the mid ureter only (p < 0.05). Hydrostatic pressure in the renal pelvis showed a rhythmic variation between 0 and 6.5 cm H2O that was nonsynchronous with respiration and also unaffected by atropine or carbachol. The 2 drugs did not change intravesical pressure. This finding does not support the hypothesis that anticholinergic drugs decrease detrusor contractility. We cannot explain this observation completely. It may be due to species differences. Another possible explanation may be relatively low bladder filling (150 to 250 ml) during these experiments. Imaging studies with X-rays revealed that peristalsis was still present 6 weeks postoperatively. The measuring catheter was placed approximately 2 cm proximal compared with its level on the day of introduction. Histological staining with hematoxylin and eosin of the harvested ureter revealed only a mild inflammatory response.

DISCUSSION

Importance of chronically instrumented animal studies versus acute experiments. A considerable number of studies on ureteral physiology that are based on in vitro or acute in vivo experiments have been published.14–17 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, eg 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 such as mercaptoacetyltriglycine (MAG-3) renography, 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 setup is similar to that used in humans for percutaneous nephrolithotripsy with a double pigtail catheter left in situ. Our detailed control studies revealed that the UUT in our animals was not affected to any significant degree by inflammation or signs of obstructive uropathy.

Extensive searches in the Medline database failed to reveal reports with an approach similar to that in 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 humans because of the long thoracic cage covering the kidney. Pigs are by nature intelligent but rooting, inquisitive and destructive animals. There-

<table>
<thead>
<tr>
<th>Experimental Condition</th>
<th>Mean Pmax ± SEM (cm H2O)</th>
<th>Mean Signal Length ± SEM (mm)</th>
<th>Mean Frequency ± SEM (min−1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Proximal</td>
<td>Distal</td>
<td>Proximal</td>
</tr>
<tr>
<td>Antagonist:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>REF-1</td>
<td>45.3 ± 1.7*</td>
<td>46.5 ± 1.7</td>
<td>26.0 ± 1.7</td>
</tr>
<tr>
<td>REF-2</td>
<td>50.1 ± 1.4*</td>
<td>48.6 ± 1.5</td>
<td>25.6 ± 1.5</td>
</tr>
<tr>
<td>Atropine</td>
<td>51.4 ± 1.4</td>
<td>50.3 ± 1.5</td>
<td>28.3 ± 1.5</td>
</tr>
<tr>
<td>Agonist:</td>
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<tr>
<td>REF-1</td>
<td>43.7 ± 1.5*</td>
<td>53.4 ± 2.0</td>
<td>29.3 ± 1.8</td>
</tr>
<tr>
<td>REF-2</td>
<td>48.2 ± 1.7*</td>
<td>55.2 ± 2.1</td>
<td>29.4 ± 2.1</td>
</tr>
<tr>
<td>Carbachol</td>
<td>49.4 ± 1.9</td>
<td>50.7 ± 2.0</td>
<td>30.1 ± 2.1</td>
</tr>
</tbody>
</table>

Pmax and peristaltic frequency in mid ureter were significantly increased during perfusion of renal pelvis with 0.25 ml per minute saline (REF-2 versus REF-1), and atropine and carbachol failed to affect ureteral peristalsis in any parameters compared with REF-2 results.

*p < 0.05.
fore, it was a great challenge to position and maintain all tunneled measuring catheters in situ during the weeks of experimentation. Despite our efforts we lost 1 catheter, as mentioned. Animal movement negatively affects the documentation of slight signals. To this end the pig socialization program with the investigator was of crucial importance to decrease the number of artifacts to a minimum and ensure the success of the experiments.

Discrepancy between in vivo and in vitro studies. Cholinergic nerve fibers are present in various densities in the UUT,\textsuperscript{13,14} They are reported to be of the muscarinic type in most species, including pigs.\textsuperscript{15} In vitro acetylcholine is reported to have an isotropic and chronotropic effect on the UUT.\textsuperscript{14–18} Since the systemic effects of cholinergic stimulation and inhibition were observed in all animals in our study, there can be no doubt that an effective biological dose of the drug was administered. Furthermore, our experimental setup was calibrated to register changes in the frequency and contraction force of ureteral peristalsis when the renal pelvis was perfused with 0.25 ml per minute saline. Therefore, our results appear to correlate with the functional relevance of the muscarinic receptors in the UUT of the conscious pig. The reason why cholinergic receptor manipulation failed to show any functional response is puzzling but the possibilities are that other regulatory circuits are superimposed on cholinergic receptor function or 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 influence effectively its function.

Presumed clinical relevance. Anticholinergic (antimuscarinic) drugs are used in clinical urology to treat bladder motor hyperreflexia and renal or ureteral colic. The mechanism of action underlying the therapy is that an antimuscarinic drug would decrease hypercontractility and spasms of the bladder and ureter. Based on our experiments this spasmylic effect does not exist. In confirmation to our knowledge no clinical evidence has ever been reported that the continuous use of anticholinergic medication for a long period for bladder hyperreflexia resulted in UUT dilation. However, the observed co-localization of muscarinic receptors with calcitonin gene-related peptide positive nerves may suggest a sensory function for muscarinic receptors.\textsuperscript{20} Therefore, anticholinergic agents may provide relief from the pain of ureteral or renal colic via this pathway. Nevertheless, we are aware that species differences may have an important role in any experimental animal model.

Conclusions

Ureteral peristalsis persisted in the initial hours after the operation on the pyelocaliceal system. Normal function was reestablished a week after manipulation. Recovery followed a gradual and hierarchic pattern from proximal to distal.

At low diuresis rates peristaltic frequency was directly related to the pyelocaliceal urine load. Although ureteral contraction force was increased in the mid ureter when the diuresis rate was increased, $P_{\text{max}}$ failed to increase in the distal perivesical ureter.

Ureteral smooth muscle motor activity at the mid and distal ureteral level was not modulated by muscarinic receptors in our chronically instrumented animal model. Therefore, the presumed spasmylic effect of anticholinergic therapy for renal colic is not supported by our findings.

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References