CHAPTER 10

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

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ABSTRACT

OBJECTIVES: The hypothesis that stimulation and inhibition of nitrergic receptors leads to a decrease and increase, respectively, of ureteric contractile function in vivo in a chronically instrumented large animal study, was tested.

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 studied before and after administration of LNNA (Nω-nitro-L-arginine, an inhibitor of nitric oxide synthase (NOS)) and L-arginine (the substrate of NOS) in comparison with control measurements with perfusion of the nephrostomy with 0.25 ml/min saline (REF).

RESULTS: Systemic effects of the agents demonstrated that functionally effective doses were used. Administration of LNNA resulted in an increase of amplitude of ureteric peristaltic force in the distal ureter and in the hydrostatic pressure in the pyelocalyceal system. L-arginine did not affect the contractility of ureter but did result in a significantly higher diuresis. The frequency of peristalsis and the length of the contracted segment of ureter were not affected by either agent.

CONCLUSION: The biological effects of NO on ureteric motility differed regionally and corresponded with the distribution of NOS-positive nerves. Inhibition of the NOS modulates the phasic contraction of the distal ureter. It also increases the tonic activity of the ureteric muscle, resulting in a higher hydrostatic pressure in the renal pelvis.
INTRODUCTION

Ureteric peristalsis originates from pacemaker activity of some smooth muscle cells located in the pyelocalyceal system $^{1-5}$. The electrical linkage of the smooth muscle cells of the ureter via gap junctions allows the propagation of a peristaltic wave. The involvement of both cholinergic and adrenergic nerves in the regulation of ureteric peristalsis is reported $^{6-10}$. There are however several experimental \textit{in vitro} and \textit{in vivo} data that suggest that non-adrenergic non-cholinergic (NANC) neurotransmitters are also involved in ureter physiology $^{7,11-13}$. Nitric oxide (NO) is reported as one of the agents playing a role in the physiology of the upper urinary tract (UUT), but its exact role in normal ureteric peristalsis still needs to be defined.

Nitric Oxide Synthase (NOS) converts arginine into equimolar amounts of citrulline and NO. Different isoforms of NOS exist. NOS-I, and -III are the so called constitutive NOS isoforms. Their activity can be stimulated by supplying high doses of the precursor arginine and inhibited by arginine derivatives such as N\textsubscript{o}-nitro-L-arginine (LNNA). NOS-II is inducible by cytokines and plays a major role in the control of inflammation. NO produced by constitutive NOS isoforms serves as a neurotransmitter in the central and peripheral nervous system. It is also relaxes smooth muscle cells and, in fact, was originally known as endothelial-derived relaxing factor. NO in urology is extensively studied as a mediator of penile erection (via vasodilatation), prostatic secretion and contraction of seminal vesicles and the ductus deferens $^{14,15}$. The involvement of NO in renal hemodynamics and in renal tubular function has also been demonstrated $^{16-18}$.

Nitric oxide synthase-positive (NOS+) nerves have been described in the urinary tract $^{3,4,11,19-21}$. Many NOS+ nerves are found in ganglia near the distal ureter and ureterovesical junction (UVJ) $^{4,19,22}$. Apparently, few NOS+ nerve cells are present in the mid ureter. Application of NOS inhibitors inhibits relaxation of the pig distal ureter \textit{in vitro}, which suggests a role as transmitter for NO in the UVJ, possibly to prevent vesico-ureteral reflux $^{11,23}$. We studied the modulatory role of NO on the mid and distal ureter in a conscious animal model to test the hypothesis that inhibition of NO synthesis would increase in the contraction force of peristalsis in the distal ureter,
whereas providing excess of the NO precursor arginine would decrease the contractility of the ureter at this level.

**MATERIALS AND METHODS**

The experimental design and procedures have been reported in detail. Permission of the local ethical committee for laboratory animals was obtained after a statistical estimate revealed that nine animals would be sufficient. Twelve female pigs were instrumented as follows. A special electronic measuring catheter to register peristaltic wave activity was implanted into the right ureter in an antegrade fashion. Tunnneled nephrostomy, venous, arterial and vesicostomy catheters were also implanted. A blank registration of ureteric peristaltic activity (REF) was undertaken during perfusion of the renal pelvis with 0.25 ml/min saline at body temperature. The effects of administration of substrate (L-arginine) or inhibitor (LNNA) of NOS were subsequently recorded. Ureteric peristalsis was visualized using a perfusion of the renal pelvis with 0.25 ml/min iodine contrast and x-ray fluoroscopic control in the sedated animal at the end of the study. Any systemic effects were registered, using blood pressure, ECG and clinical monitoring of side effects as parameters. The results were statistically analyzed and reported as mean ± SEM.

LNNA (50 mg/kg) and L-Arginine (180 mg/kg) were administered intravenously to inhibit and to stimulate NO synthesis. LNNA was dissolved in 500 ml acidic saline (pH 5), subsequently neutralized to pH 7, and passed through an anti-microbial filter to prepare a parenteral injection fluid. To compensate for the volume load of 500 ml that was needed to dissolve LNNA, the pigs were fasted for a 12-hours period before the experiment. LNNA experiments were planned as the last experiment in our series, as the half-life of LNNA is long. The L-Arginine was already prepared for human parenteral use.

*Care and follow-up of animals*

Daily each animal was systematically examined. In all animals, urinary leak 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 and to reduce animal stress to minimum during the study.

RESULTS

Qualitative description of the registered peristalsis
The criteria used to distinguish a peristaltic wave were as described previously\(^\text{24}\). Registration was possible in every experimental session. In two pigs, only a single channel registration was possible, because of technical failure. One pig destroyed the measuring catheter. The animal was re-operated to place a new catheter.

X-ray imaging studies revealed that peristalsis was present in all pigs at 6 weeks postoperatively. The catheter was displaced proximally by about 2 cm relative to its initial position.

Quantitative description of the registered peristalsis
The variation of \(P_{\text{max}}\) (maximal amplitude of peristaltic phasic contraction) and frequency of ureteric peristalsis in REF and after administration of LNNA or L-arginine are illustrated in Figure 1.
FIGURE 1: Effect of modulation of NO synthesis on ureteric peristalsis. Panel A illustrates the effects of LNNA and L-arginine in the distal and mid-ureter on $P_{\text{max}}$ in comparison with REF. In panels B and C, the length of the contracted segment and the peristaltic frequency, respectively, are illustrated.
Relative to REF (43.2 ± 1.8 cm H₂O), Pₘₐₓ (72.3 ± 2.1 cm H₂O) is increased only in the distal ureter of the LNNA group (P< 0.05). Perfusion of 0.25 ml/min saline through the nephrostomy causes a small, but significant (REF: 52.7 ± 1.7 cm H₂O) increase of Pₘₐₓ only in the mid ureter of the LNNA (Pₘₐₓ: 51.3 ± 1.5 cm H₂O) group. The peristaltic frequency (1.7± 0.2 min⁻¹ in LNNA group and 2.1 ± 0.2 min⁻¹ in L-arginine group) and length of the ureteric contraction during the peristalsis were not affected by LNNA (REF: 29.0 ± 1.8 mm in mid ureter and 28.8 ± 1.0 mm in distal ureter; LNNA group: 29.5 ± 1.7 mm in mid ureter and 30.0 ± 1.3 mm in distal ureter) or L-arginine (REF: 28.5 ± 1.9 mm in mid ureter and 29.9 ± 1.6 mm in distal ureter; L-arginine group: 29.3 ± 1.9 mm in mid ureter and 29.6 ± 1.5 mm in distal ureter).

LNNA increased hydrostatic pressure in the renal pelvis significantly, showing a rhythmic variation between 12 and 18.5 cm H₂O (control: 0 to 6.5 cm H₂O, P<0.05) that was not synchronous with respiration. Intra-vesical pressure was not affected to a significant degree by LNNA. L-arginine again did not have any significant effect on hydrostatic pressure in the renal pelvis or in the bladder.

**Systemic side effects of the investigated drugs**

L-arginine and LNNA caused a decrease in heart rate from 81 ± 4 min⁻¹ in controls to 75 ± 4 min⁻¹ (not significant) and an increase to 120 ± 2 min⁻¹; P<0.05), respectively. Blood pressure was decreased from 130/90 ± 6/7 mmHg in the control group to 120/90 ± 8/5 mmHg in the L-arginine group and increased to 300/190 ± 9/7 mmHg in the LNNA group (P<0.05). The average diuresis of the pigs in the LNNA, L-arginine and control groups was 74 ± 2 ml/hour, 82 ± 1 ml/hour and 70 ± 1 ml/hour, respectively (P< 0.05 for the L-arginine group). No general symptoms were observed after L-arginine administration. Seven of the 11 pigs developed a nystagmus after administration of LNNA. The severe hypertension after the LNNA treatment lasted 3 days.
DISCUSSION
A chronically instrumented large animal model was developed to circumvent criticisms on acutely performed experiments studying ureter physiology. The effects of this chronically instrumented model was reported earlier.

Effect of LNNA and L-arginine on renal blood flow and diuresis
Ureteric peristalsis in vivo is not only dependent to the excitatory status of the smooth muscle cells in the upper urinary tract UUT, but also on the rate of diuresis. NOS blockade resulted in vasoconstriction and a decreased renal blood flow \(^{16-18}\), whereas exogenous NO resulted in vasodilatation and an increased renal blood flow \(^{25}\). Renal blood flow determines the rate of diuresis and thus indirectly the frequency of ureteric peristalsis.

In this study, inhibition of NO synthesis with LNNA resulted in a malignant hypertension that lasted approximately 3 days. The heart rate was also increased. However, the diuresis was not significantly different from the control group, perhaps because the increased blood pressure neutralizes the effects of vasoconstriction in the renal vascular bed. L-arginine did result in a significantly higher diuresis rate due to its vasodilatory effect, increasing renal blood flow and glomerular filtration rate without significantly changing blood pressure. As far as we are aware, these findings have not been reported previously in such a study.

Effect of LNNA and L-arginine on upper urinary tract motility
NO is argued to be inhibitory on upper urinary tract motility \(^{26-27}\). In vitro, NO donors decrease the spontaneous and induce contraction of smooth muscle cells of the pyelocalyceal system in a dose-dependent fashion \(^{26}\). A negative effect of NO on the tonic contraction of the human ureter and of experimental animals is also reported \(^{27-31}\). These effects are probably mediated via NOS-III dependent NO synthesis in the porcine and human pyelocalyceal system \(^{26-27}\).

Systemic side effects of LNNA and L-arginine were observed, demonstrating that biological and physiological effects on the ureter could be expected. Perfusion of the renal pelvis with 0.25 ml/min saline through the nephrostomy was undertaken to compensate for the
possible hypo-diuretic effect of LNNA and L-arginine. Administration of LNNA resulted in a significant increase of $P_{\text{max}}$ in the distal ureter, that is, where the highest concentration of NOS+ nerves was reported in the literature. LNNA did not increase the $P_{\text{max}}$ in the mid-ureter. L-arginine on the contrary was not able to reduce the $P_{\text{max}}$ in either the mid- and distal ureter. The pre-existence of local endogenous NO excess may explain this finding.

The distal ureter may be kept in a relaxed status by the local abundance NOS+ nerves. A more powerful contraction of this part of the ureter may be produced by a temporary inhibition of NO synthesis. Constitutive NOS isoforms are dependent for their activity on intracellular $\text{Ca}^{2+}$, limiting the activity of these NOS isoforms to $\text{Ca}^{2+}$ transients. The short half-life of NO further assures that relaxation of the ureter stops after the passage of the peristaltic wave.

LNNA and L-arginine did not affect peristaltic frequency and the length of the contracted segment of the ureter during peristaltic activity. These findings are at variance with earlier data, which suggested that spontaneous pyelocalyceal activity was affected by NO in vitro 26-27. This discrepancy may be explained by the compensatory effect of alteration in diuresis and/or rate of perfusion of saline through the nephrostomy and its direct effect on the peristaltic frequency of the ureter. The length of contracted segment depends on the duration of contraction and is independent of state of NO activity.

Hydrostatic pressure in the pyelocalyceal system was increased in LNNA group. This finding supports the supposition that blockade of NO synthesis will increase the tonic activity of ureteric muscle. However, because of the severe systemic side effects of LNNA, blockage of NO synthesis is not (yet) a modality to treat vesico ureteral reflux. L-arginine failed to alter the hydrostatic pressure in the pyelocalyceal system.

**CONCLUSION**
The biological effects of NO on ureteric motility vary regionally and correspond with the distribution of NOS. Systemic inhibition of NO synthesis results in a significant rise in tonic and phasic ($P_{\text{max}}$ only in the distal ureter) contraction force of the ureter, whereas supplying extra substrate (L-arginine) does not reduce the phasic and tonic
contractility of the ureter. These findings indicate that the distal ureter is inhibited by abundant local NOS activity and is regulated phasic inhibition of the NOS+ nerves. From our model, the fundamental role of NO metabolism in controlling distal ureter motility seems to be explained.

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REFERENCES


Chapter 10: effect of Nitrergic stimulation and inhibition


