"The Effect of Diabetes mellitus on Urethral Neuromuscular Function"

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The intent of this project was to understand the effects of Type I diabetes mellitus (DM) on the behavior of lower urinary tract smooth musculature. In year one, we developed an *in vivo* video urodynamic technique which allows for the determination of the contributions by the multiple smooth muscle components of the LUT to the physiological outcomes of bladder filling and storage, on the one hand, and voiding by the entirety (bladder and urethra) on the other hand. We also developed a novel method for urethral biomechanical testing involving video multiple position measurement of *ex vivo* whole mount urethras. In year two, we pursued the pharmacological challenges to non-voiding bladder activity during video urodynamics and also began tissue processing for histology.

The Nature of Non-Voiding Contractions in the Rat: A Pharmacological Investigation using a Novel Video Urodynamics Technique

Introduction

Non-voiding contractions (NVC) during filling are often utilized as surrogates for urgency in preclinical studies. Recent results from our laboratory, presented here last year, suggested that some NVC may be a normal part of the filling mechanism, directing urine from the base to the dome in a peristaltic wave. These filling contractions often appear shortly after the initiation of filling via the ureter, and we have hypothesized that they may arise as a continuation of the electrical activity propagated down the ureters, possibly coordinated by the trigone.

Another source of NVCs is the dome of the bladder, and this type of NVC characteristically appears toward the end of the filling cycle, if they are to appear at all, as a response to the inflation of the dome by the base-to-dome filling contraction. These visually evident dome muscular contractions have been referred to by others when referring to cystometric traces as prodromal contractions, and in our hands they had the appearance of a strongly coordinated detrusor contraction, resulting in both bladder neck and proximal urethral dilation. Thus, we hypothesized that it is these latter NVCs which are the true surrogates for urgency, due to their reciprocal inflation of the bladder neck and proximal urethra. We further hypothesized that these contractions arose from a spinal reflex, which gave the appearance of a micturition reflex but without the relaxation of the external urethral sphincter. We sought to test this hypothesis and further discriminate between base-to-dome contractions and dome-to-base NVCs using pharmacological manipulation of autonomic function. It was important for us to conduct these experiments first in control animals in order to determine the mechanisms involved in the generation of non-voiding contractile activity in normal rats prior to testing DM animals.

Methods

The lower urinary tracts of urethane anesthetized (1.2 g/kg) female SD rats were exposed by midline

laparotomy and pubic symphysis reduction or removal (Figure 2). The animals were mounted on a frame and their abdomens filled with mineral oil. Cystometric investigation utilized transureteral filling at a physiological flow rate (0.02-0.04 ml/min) and a static transurethral catheter for pressure recording. Video capture of bladder motility was performed using a high speed video camera with a LabView frame grabber program. Pressure recordings were made using LabChart. Under these conditions, numerous non−voiding pressure waves during each filling cycle and their associated bladder contractile activity are readily evident along with subsequent micturition events (Figure 1). After several control cycles, we sequentially administered atropine (ATR; 0.4-0.8 mg/kg), HEX (25-50 mg/kg), a beta 3 adrenergic agonist (CL−316,243; 100 ug/kg) and/or isoproterenol (ISO; 100 ug/kg; Figure 1).

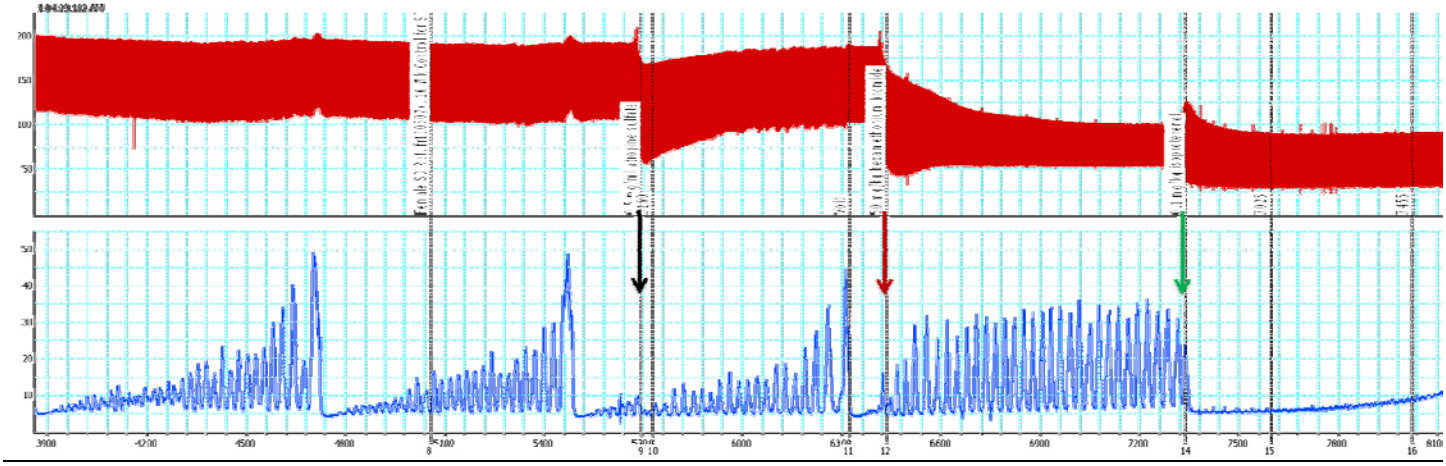

Figure 1. Cystometric trace during video urodynamic study of normal female rats before and after drug treatment. The top trace is carotid artery pressure, bottom trace is transurethral bladder pressure. Following control micturition cycles, atropine was administered (black arrow), followed by hexamethonium (red arrow) and finally isoproterenol (green arrow). Only beta adrenergic agonists were capable of quieting the bladder NVCs in this preparation.

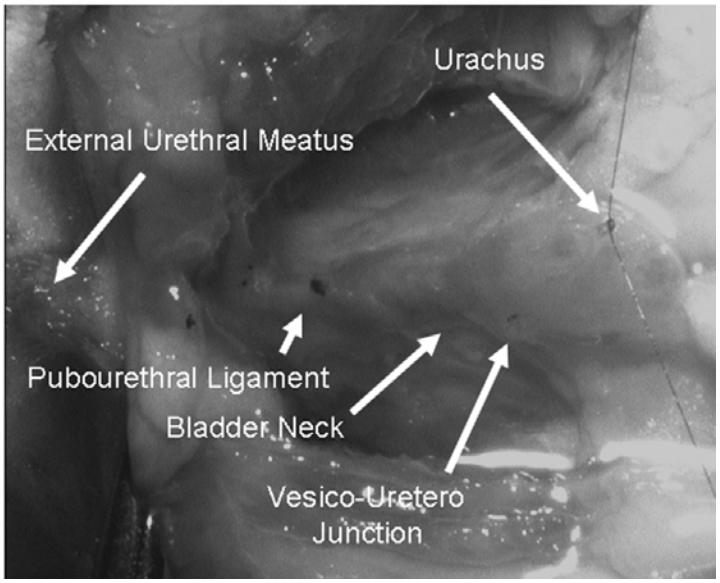

Figure 2. Exposure of the rat lower urinary tract for video urodynamic recording.

Results

ATR had little effect on either filling contractions (a.k.a. “Initial NVCs”) or apparent prodromal contractions (Figures 1 and 3), even though it increased compliance (data not shown). Continued bladder activity was verified both by cystometric trace and video. Ganglionic blockade by HEX resulted in a dramatic increase in amplitude and area of NVCs. CL and ISO resulted in an initial almost total abolition of contractile activity, and thus NVCs. It should be noted that only one of the animals demonstrated an apparent full coordinated contraction of the dome during prodromal contraction. Of note, when prodromal activity became apparent, it appeared to result in a decrease in baseline bladder pressures.

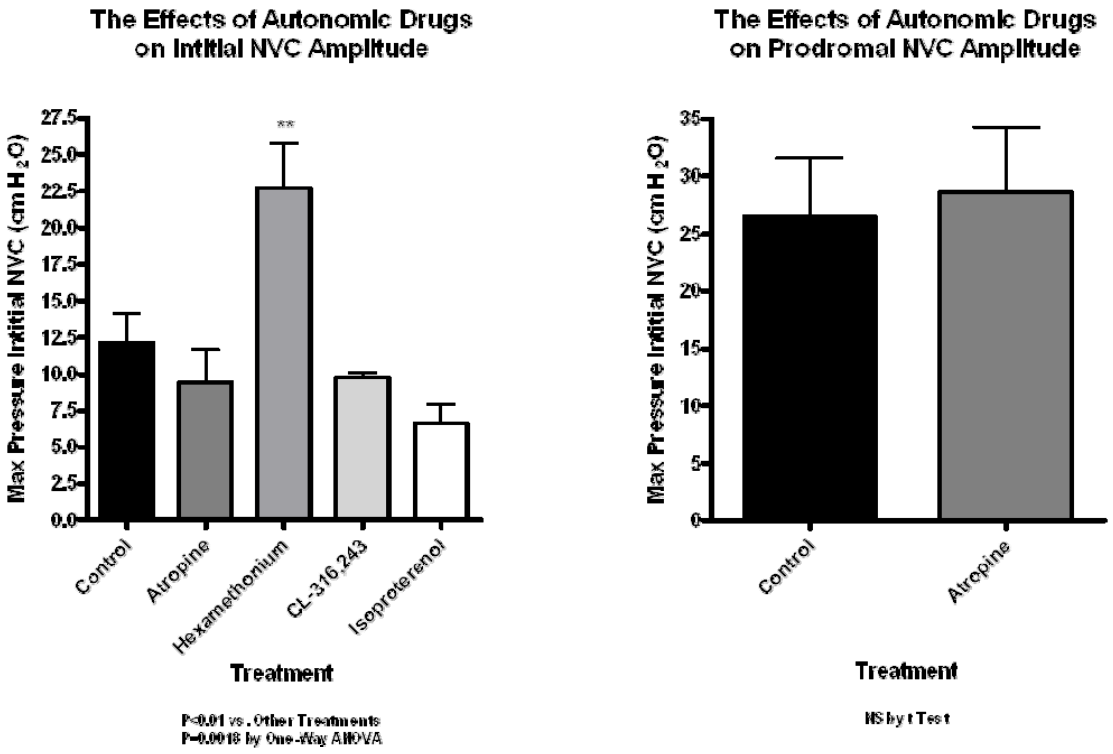

Figure 3. The effects of pharmacological treatment on NVC amplitudes. Left graph illustrates the effect of autonomic drugs on initial, filling NVCs, while the right illustrates the lack of effect of ATR on prodromal NVCs

Conclusion

The results of the current study suggest that autonomic efferent pathways are not responsible for producing filling NVCs in this preparation, and in fact the sympathetic nervous system appears to keep this activity in check, as evidenced by HEX enhancement and beta agonist quieting of NVCs. That muscarinic blockade was ineffective in eliminating filling NVCs and that ATR and HEX seemed to have no effect on prodromal NVCs of this series suggests the prodromal contractions seen here were not neurogenic in origin. However, it must be stated that these prodromal contractions were not visually similar to those which preceded the micturition-like, fully coordinated dome contractions in previous experiments. It may be the case that these differences in coordination represent two different mechanisms for NVC production. Thus, we are unable to state conclusively that we have shown all prodromal dome-to-base contractile activity to be non-neural. Future studies will attempt

to elicit the putatively neurally-mediated reflex contractions with bladder irritation in order to test whether ATR and HEX have any effect on these more coordinated, micturition-like dome contractions.

Thus, we now believe that the bladder can be a rather noisy storage unit, with myogenic contractile activity directing urine from the base to the dome, along with myogenic dome activity in some as the dome is expanded, some of which gives the appearance of regional coordination but overall “mixing”, while in other cases there appears to be coordination mimicking a micturition event without the accompanying EUS silence.

It will be of great interest to determine whether the rugated appearance contracture state seen in DM animals (Figure 4) will be neural (atropine and/or hexamethonium sensitive) or myogenic in origin, and if the latter, whether they will respond to beta adrenergic stimulation. We currently have STZ treated and control rats in the pipeline for this investigation.

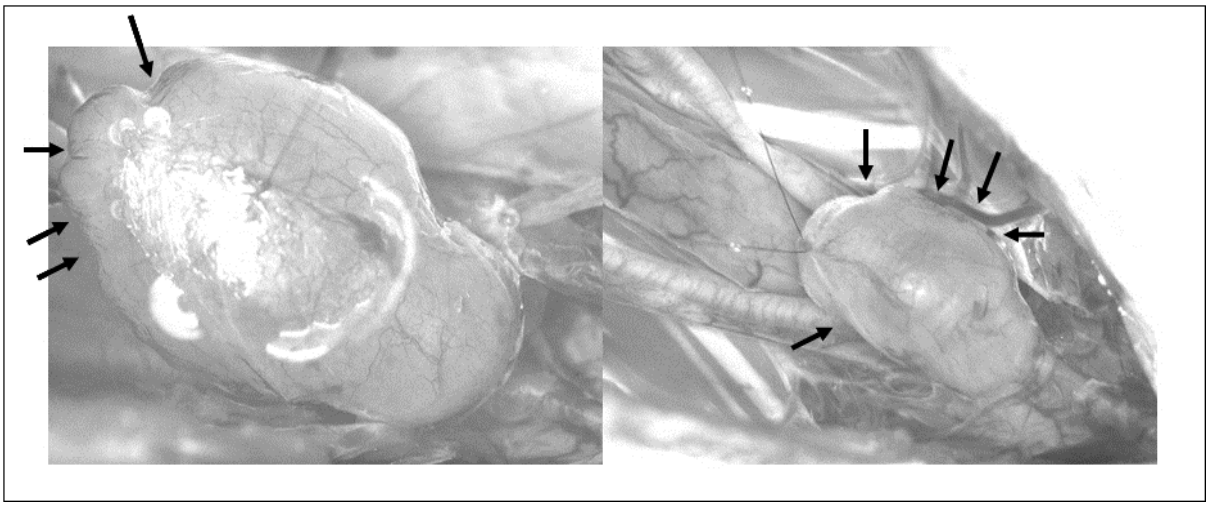

Figure 4: Two examples of rugated appearance of bladder domes from DM rats. Left panel is from 5 Wk DM animal near bladder capacity, right panel is from 10 wk DM at early stage of filling. This highly unusual contractile pattern has only been seen with DM rats.

Microscopic Anatomical Studies of LUT Muscles in Vehicle treated and STZ-induced DM animals

We have divided the lower urinary tracts of 55 rats (~50% vehicle control and STZ-DM across 20 weeks of treatment at 3, 6, 9, 12 and 20 weeks) into bladder and urethra. We further divided the urethras into four regions - proximal-proximal, distal-proximal, proximal-distal and distal-distal. From these, each has been sectioned by alternating 20 micron thick sections alternately onto 6 slides for a total of eight sections per slide. The resultant 1, 320 slides are being processed for immunofluorescence staining of neuronal and non-neuronal markers. We are still in the process of both staining and microscopic evaluation.

Conclusions

Work will continue to test in vivo hypotheses and process tissues for histology. A wealth of information is expected, and these results have already resulted in the formation of a Workshop at the 2011 International Continence Society, Chaired by the PI, on non-neural bladder activity in health and disease.