

Vardenafil decreases bladder afferent nerve activity in unanesthetized decerebrate spinal cord-injured rats

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OBJECTIVES

- Several experimental studies have suggested that NO released in the bladder from the urothelium and/or suburothelial afferent nerves may modulate the micturition reflex by reducing the excitability of bladder afferents.
- Moreover, it was shown that a single dose of vardenafil improved urodynamic parameters in SCI patients with most significant improvement in reducing maximum detrusor pressure and detrusor overactivity and increasing maximum cystometric capacity suggesting an effect of vardenafil beyond direct relaxation of bladder smooth muscle.

In order to evaluate the effects of vardenafil on bladder afferent nerve activity-induced NDO in SCI rats, we aimed to:

- evaluate detrusor overactivity and modifications of urodynamic parameters with cystometry experiments
- evaluate bladder afferent nerve firing (BANF) in response to bladder filling with electrophysiological experiments.

MATERIALS & METHODS

Complete spinal cord transection

Complete T7-T8 spinalization was performed in 15 female adult Sprague-Dawley rats (>250 g).

In order to prevent urinary tract infection (UTI), the animals were treated postoperatively with antibiotics. Urinary bladders were manually expressed by Credé maneuver first 3 times daily then 2 times daily until an abnormal micturition reflex was totally established.

Surgical preparation

At 21-29 days post SCI, rats were anesthetized with isoflurane to place iv and bladder catheters and perform the decerebration to avoid confounding effect of anesthesia, according to a published method (Yoshiyama & de Groat, *Neurosci. Lett.* 2007). Experiments started at least 2 hours after decerebration.

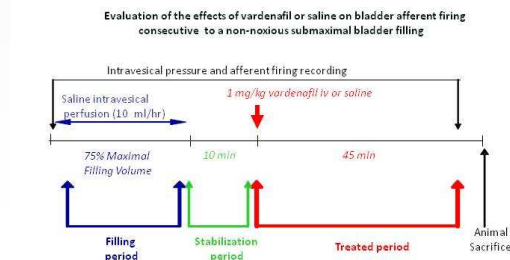
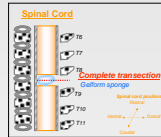
The urethra was ligated to be able to accurately compare afferent nerve activity with intravesical pressure. For pelvic nerve (PN) electrical stimulation, the left pelvic nerve was placed on a bipolar stimulation electrode sealed with nonreactive Wacker gel (Wacker Chemie, Germany). Then, fine filaments were dissected from the L6 dorsal roots and placed across a bipolar electrode. Signals were recorded continuously, amplified and filtered (10.000x amplification, 0.1-5 kHz bandpass filter) (model 1700 AC amplifier, A-M Systems, USA) to record spiking activity denoting action potentials from single-unit fibers. Afferent nerve fibers originating from the bladder were identified by electrical stimulation of the pelvic nerve and by bladder distension.

Cystometric investigation

First, bladder filling (10 ml/hr) was performed to determine the maximal bladder filling volume (BFV).

In a second step, the bladder was filled up to 75% of its maximal filling volume (non-noxious BFV) and allowed to stabilize both in terms of contractions and afferent firing. Then, vardenafil (1 mg/kg, n=7) or saline (n=8) were delivered i.v. and the recording continued for another 45 min (treated period). NVC were characterized by the force generated (AUC, in mmHg.s) while the frequency of BANF (spikes/s) was determined during 45 minutes. All results were expressed as % of baseline and compared using two-way ANOVA.

At the end of the experiment, the animal was sacrificed with an overdose of urethane (1.4 g/kg).

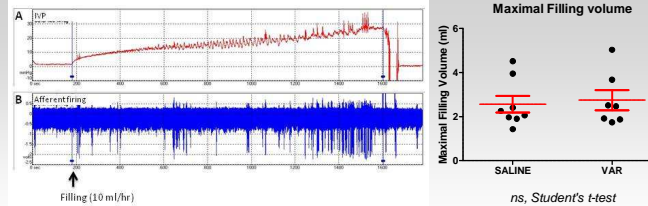


Note: Although initially, bladder afferent fibers were classified according to their conduction velocity into Aδ and C fibers, more recent reports have shown that the properties of mechanoreceptors sensitive to bladder filling are not different between both types of afferents in rats (Sengupta & Gebhart, *J Neurophysiol* 1994; Shea et al., *J Neurophysiol* 2000; Gillespie et al., *BJU Int* 2009). Therefore, such differentiation was not taken into account for the results.

RESULTS

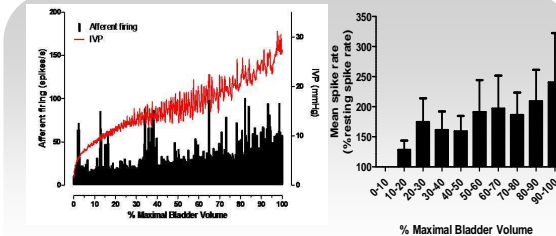
Determination of maximal bladder filling volume (BFV)

Mean maximal bladder filling volume



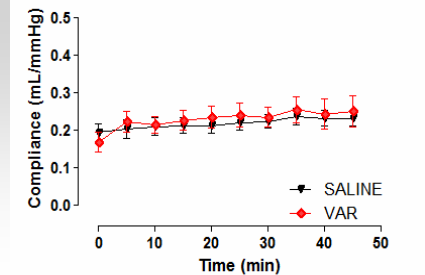
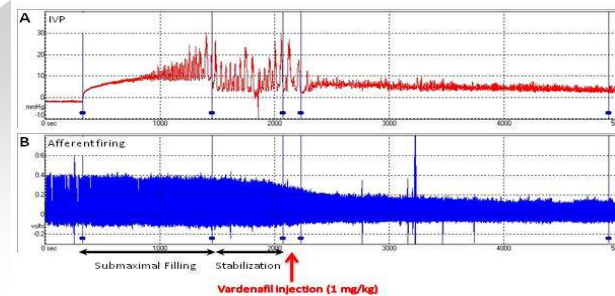
- Intravesical pressure changes during filling consisted of a slowly increasing component with transient "non-voiding" contractions superimposed, until fullness of the bladder was achieved
- BFV did not differ between those rats that later received saline or vardenafil i.v.

Relationship between BANF and intravesical pressure and volume in unanesthetized decerebrate SCI rats

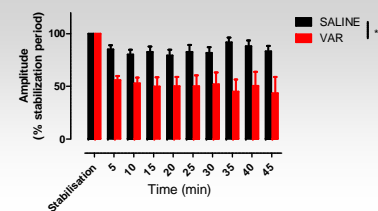


- BANF rate increased with bladder filling

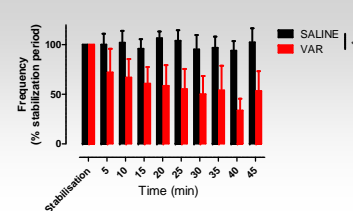
Effect of vardenafil iv (1 mg/kg) on NVC and BANF in decerebrate unanesthetized SCI rats



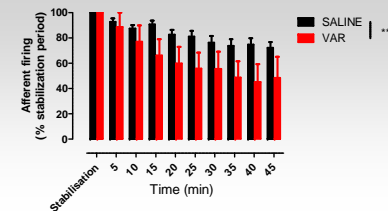
Amplitude of non-voiding bladder contractions



Frequency of non-voiding bladder contractions



Effect of vardenafil on bladder afferent firing



CONCLUSIONS

- Vardenafil iv 1 mg/kg significantly inhibited non-voiding contractions induced by submaximal filling of the bladder and concomitantly significantly reduced bladder afferent nerve firing activity in unanesthetized decerebrate SCI rats.
- These findings may give us a new insight into the mechanism of action of PDE5 inhibitors at improving urodynamic parameters in SCI patients, or even storage symptoms in men with LUTS /BPH.