Abstract # 128

Nitric oxide/cGMP signalling mediates an inhibitory action on sensory pathways of the micturition reflex in the rat

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PELVIPHARM

OBJECTIVES

➢Overactive bladder (OAB) can be associated with an hyperexcitability of bladder afferent fibers. In particular, C-fibers, normally silent, can become hyperexcitable under pathophysiological conditions and therefore could be responsible for inducing bladder hyperactivity. Several studies have suggested that nitric oxide (NO) or its downstream signalling could modulate the micturition reflex by reducing the excitability of bladder afferents.

We aimed to evaluated the role of each of the key components of the NO/cGMP signalling pathway i.e NOS, NO, sGC, cGMP and PDE5 on the micturition reflex in a rat experimental model of bladder hyperactivity due to C-fiber activation by capsaicin.

MATERIALS & METHODS

✓**Animal preparation**

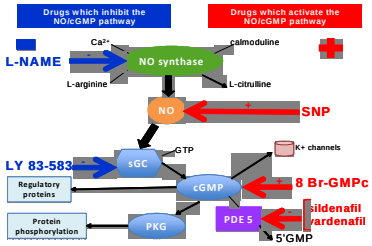
In all, 75 female adult Sprague-Dawley rats (weighing 250-275 g; Elevage Janvier, France) were used. The protocol for this study complied with the animal protection legislation for animal studies in experimentation and all other applicable laws and regulations in force in France.

The rats were anesthetized with isoflurane (0,8-1%, Centravet, France). The left carotid artery and the right jugular vein were catheterized with a polyethylene catheter (PE-10: 0,28 OD) filled with heparinized saline (25 UI/ml) for mean arterial pressure monitoring and for drug injection into the bloodstream, respectively. Ureters and bladder were exposed via a midline abdominal incision. Ureters were transected and ligated distally to prevent bladder filling with urine during experiments and a polyethylene catheter (PE-50: 0,58 OD) was inserted within the bladder dome. The catheter was connected to a pressure transducter (Elcomatic EM 750, UK) for bladder pressure monitoring and to a syring-pump KDS-200 (Kd Scientific, Phymep, Paris) allowing bladder perfusion. The bladder pressure was recorded continuously using a specific data acquisition software (Elphy, CNRS, France).

✓**Cystometry experiments.**

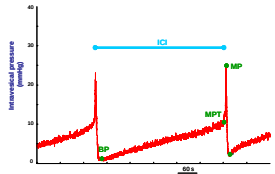
The bladder was continuously perfused (50 µl/min) with saline during a stabilization period of 60 min to check the quality of the recording and the frequency of micturition events. The perfusion was then switched to 30 µmol/l capsaicin with the same rate. A control period of 45 min was recorded. Then, drugs or vehicle were delivered by intravenous (i.v) route (60 µl/min) and cystometrogram was recorded during 60 min (treated period). The drugs investigated were:

- sodium nitroprusside (**SNP**) 0.1 mg/kg, a NO donor;
- **8-Br-cGMP**, 10 mg/kg, a cGMP soluble analogue,
- **sildenafil** 3mg/kg and **vardenafil** 1 mg/kg, two PDE5 inhibitors
- **LY-83 583** 1 mg/kg, a guanylate cyclase inhibitor.
- NG-nitro-L-arginine methyl ester (**L-NAME**), 10 mg/ml, a NOS inhibitor, intravesical administration was performed concomitantly with capsaicin



✓**Data and statistical analysis.**

During cystometry experiments, the intercontraction interval (ICI), baseline pressure (BP), micturition pressure threshold (MPT), voided volume (VV) and maximal pressure (MP) were measured. The parameters of the last 15 min of the control period were averaged and used as baseline values. During the treated period, the parameters were averaged every 15 min. All data values were expressed as mean plus or minus standard error of the mean and were averaged per treatment group. Results were expressed as a percentage of baseline values. The comparison of the effect of drugs was performed with a two-way ANOVA statistical analysis followed by Bonferroni post hoc test. In case of interaction between the two factors (drug and time) in the two way ANOVA analysis, a modified student's t-test with the Bonferroni adjustment for multiple comparisons was performed. P values < 0,05 were considered significant. Statistical analysis was performed with GrapPadPrism® 5.03 software.



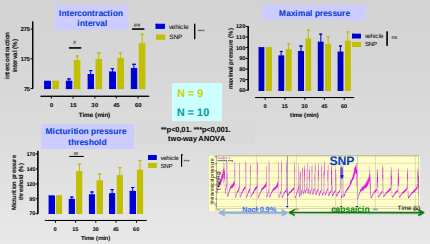
Representative cystometrogram in an anesthetized rat illustrating the parameters analysed from such experiment.

✓**Drugs and chemicals.**

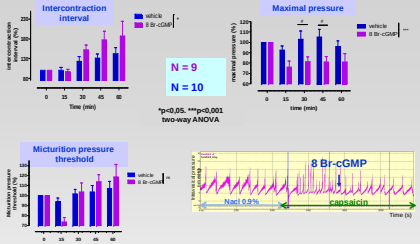
All drugs and chemicals were purchased from Sigma (Saint-Quentin, Fallavier, France), except vardenafil and sildenafil which were purchased from Alsachim SAS (Strasbourg, France) and LY-83 583 from calbiochem (Lyon, France). LY-83 583 and capsaicin were prepared in dimethyl sulfoxide (DMSO 10%). Other drugs were prepared in saline solution (NaCl 0,9%).

RESULTS

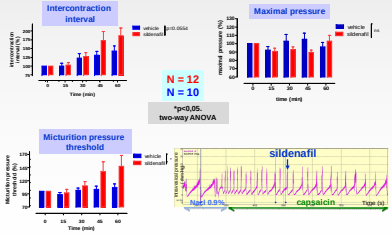
Effect of SNP



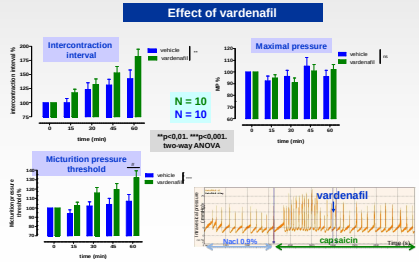
Effect of 8Br-cGMP



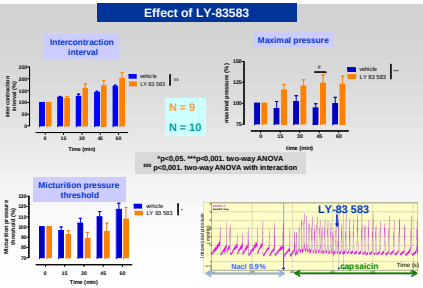
Effect of sildenafil



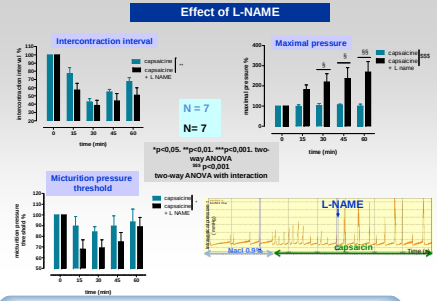
Effect of vardenafil



Effect of LY-83583



Effect of L-NAME



	Urodynamic parameters related to:				
	Voiding contractions *effect on efferent pathway*		The filling phase *effect on sensory pathway*		
	MP	VV	ICI	MPT	BP
Drugs which activate the NO/cGMP pathway: SNP	-	↑	↑	↑	-
Drugs which activate the NO/cGMP pathway: 8 Br-cGMP	↓	-	↑	↑	↓
Drugs which activate the NO/cGMP pathway: sildenafil	-	-	↑	↑	-
Drugs which activate the NO/cGMP pathway: vardenafil	-	-	↑	↑	-
Drugs which inhibit the NO/cGMP pathway: LY-83 583	↑	↑	-	↓	↑
Drugs which inhibit the NO/cGMP pathway: L-NAME	↑	-	↓	↓	-

CONCLUSIONS

- Compounds activating the NO/cGMP pathway inhibited bladder hyperactivity induced by capsaicin whereas compounds inhibiting the NO/cGMP pathway increased bladder hyperactivity induced by capsaicin.
- These results indicate that the NO/cGMP signalling pathway is involved in the regulation of the micturition reflex in a pathophysiological model of bladder hyperactivity with a mechanism of action on both the sensory and the motor components of the micturition reflex.
- This could support the potential development of NO/cGMP pathway modulators for the treatment of OAB.