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

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OBJECTIVES

» Overactive bladder (OAB) can be associated with an hypersexcitability of bladder afferent fibers. In particular, C-fibers, normally silent, can become hypersensitive 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 evaluate the role of each of the key components of the NO/cGMP signalling pathway i.e. NOS, NO, cGMP and PDEs 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 anesthetised with isoflurane (5-8%, Intervet, France). The left urethral orifice and the right jugular vein were catheterised with a polyethylene catheter (PE-10 0.38 OD) filled with heparinised saline (25 U/ml) for mean arterial pressure monitoring and for continuous infusion into the bloodstream, respectively. Urinets and bladder were exposed via a midline abdominal incision. Urinets were transected and ligated tightly to prevent bladder filling with urine during experiments and a polyethylene catheter (PE-50 0.56 OD) was inserted within the bladder dome. The catheter was connected in a pressure transducer (Statham MP 70, USA) for bladder pressure monitoring and to a spring-pump HDS-200 (IdScientific, Phymas, Paris) allowing bladder perfusion. The bladder pressure was recorded continuously using a specific data acquisition software (Ephy, CNSF, France).

Cytometry 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 μl/min capsaicin with the same rate. A control period of 45 min was recorded. Then, drugs or vehicle were delivered intravesically (i.v) route (10 μl) and cytometry 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;
- L-NAME, 10 mg/ml, an NO synthase inhibitor;
- L-arginine methyl ester hydrochloride (L-NAME), 10 mg/ml, a NOS inhibitor; intravesical administration was performed concurrently with capsaicin.

Data and statistical analysis.

During cytometry 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 multiple comparison test was used. A Bonferroni adjustment for multiple comparisons was performed. P values < 0.05 were considered significant. Statistical analysis was performed with GraphPadPrism 5.03 software.

Drugs and chemicals.

All drugs and chemicals were purchased from Sigma (Saint-Quentin Fallavier, France), except vardenafil and sildenaﬁl which were purchased from Arixontam SGS (Strasbourg, France) and LY 03 583 from calbiochem (Lyon, France). LY 03 583 and capsaicin were prepared in dimethyl sulfoxide (DMSO 1%). Other drugs were prepared in saline solution (NaCl 0.9%).

RESULTS

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.