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 hyperexcitability of bladder afferent fibers. In particular, C-fibers, normally silent, can become hyperexcitable under pathophysiologic conditions and therefore 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 Le 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.

RESULTS

- Animal preparation
  - In all, 75 female adult Sprague-Dawley rats (weighting 200-275 g; Bergeres-Jonvilliers, 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 (0.5-1%). Cervical, femoral, and the right jugular vein were cannulated with a polyethylene catheter (PE-10: 0.28 OD) filled with heparinized saline (25 IU/ml) for mean arterial pressure monitoring and for drug injection into the bladder. The catheter was connected to a pressure transducer (Statham EM 755, UK) for bladder pressure monitoring and to a syringe pump (KDS-200 Igil Scientific, France) for delivering bladder perfusion. The bladder pressure was recorded continuously using a specific data acquisition software.
- 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 micturitional 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 by intravesical (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-BrcGMP, 10 mg/kg, a cGMP soluble analog.
  - sildenafil (5 mg/kg) and vardenafil (1 mg/kg), two PDE5 inhibitors
  - LY-83 583 1 mg/kg, a guanylate cyclase inhibitor.
  - 8-NH₂-bromo-L-arginine methyl ester (L-NAME), 10 mg/kg, a NO-inhibitor. Intravesical administration was performed concomitantly with capsaicin.

- Data and statistical analysis
  - During cystometry experiments, the intravesical pressure (IVP), baseline pressure (BP), micturition pressure (MP), 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 by a two-way ANOVA followed by Bonferroni post hoc test. In case of interaction between factors, a new ANOVA was performed with the Bonferroni adjustment for multiple comparisons. Significance was considered significant. Statistical analysis was performed with GraphPadPrism™ 5.02 software.

- Drugs and chemicals
  - All drugs and chemicals were purchased from Sigma (Saint Quentin Fallavier, France), except vardenafil and sildenafil which were purchased from Abcrim SAS (Brussels, France) and LY-83 583 from calbichem (Lyons, France). LY-83 583 and capsaicin were prepared in dimethyl sulfoxide (DMSO 1%). Other drugs were prepared in saline solution (NaCl 0.9%).

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.