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Dinis P[1], Charrua A[2], Avelino A[2], Yacoob M[3], Nagy I[4], Cruz F[2]
1. Department of Urology, Hospital S. João, 2. Institute of Histology and Embryology, Faculty of Medicine of Porto, Portugal, 3. Novartis Institute for Medical Research, London, UK, 4. Imperial College, London, UK

ANANDAMIDE, AN ENDOGENOUS VR1 (TRPV1) LIGAND, INCREASES DURING BLADDER INFLAMMATION AND CONTRIBUTES TO PAIN AND DETRUSOR HYPERACTIVITY. AN EXPERIMENTAL STUDY IN THE RAT.

Aims of Study

The vanilloid receptor (VR1) plays a pivotal role during bladder inflammation, namely in hyperalgesia (1) and hyperactivity (2). However, the mechanism of VR1 activation during chronic bladder inflammation is poorly understood, in part due to the unawareness of an endogenous substance that might activate VR1 in the same way as capsaicin does. This well-known exogenous VR1 ligand induces pain and an immediate enhancement of the micturition reflex when applied to the bladder (3). Recently anandamide (ANA), a lipid synthesized from arachidonic acid by macrophages and vascular endothelium (4) was shown to activate VR1 (5). Since ANA is converted into arachidonic acid, a pro-inflammatory molecule that increases in inflamed tissues, we decided to investigate if (i) ANA concentration increases in inflamed bladders and (ii) whether it induces pain and detrusor hyperactivity when applied to normal bladders. As ANA is also known to activate the cannabinoid receptor subtype 1 (CB1) expressed in the bladder (6), the effect of exogenous ANA was also investigated in the presence of a specific CB1 receptor antagonist (SR141716A).

Methods

To evaluate the concentration of ANA in inflamed bladders, cystitis was induced in adult female Wistar rats by intraperitoneal injections of cyclophosphamide (one injection of 200mg/kg of body weight or 3 injections of 75 mg/kg every three days). Bladders were harvested under anesthesia at 4 hours, 24 hours, 3 days and 7 days after the onset of inflammation and immediately frozen at –80ºC. Bladders of naïve or saline injected rats were used as controls. The anandamide concentration was determined by mass spectrometry.

To evaluate the effect of ANA in bladder reflex activity, adult female rats were anesthetized with urethane and the bladder exposed through a midline abdominal incision. Saline was infused at 6 ml/h through the bladder dome while the urethra was kept opened. Body temperature was maintained at 36-37ºC. After a period of stabilization, ANA (Tocris, UK) in 1, 5, 10, 50 and 100 μM solutions in saline were applied sequentially on the serosal surface of the bladder (10 minutes for each solution) and the number of reflex bladder contractions counted in each period. These experiments were repeated in rats treated 24 hours before with 10 nM intravesical RTX, or with 50 μM CPZ and 50 μM SR141716 immediately before ANA application.

To evaluate the nociceptive effect of ANA, 0.5 ml of saline, 50 μM ANA or 50 μM ANA+50 μM CPZ were instilled through the urethra of halothane anesthetized adult female Wistar rats and left in the bladder during 30 minutes. In addition, 50 μM ANA was also applied in rats treated 24 hours before with intravesical 100 nM RTX. Two hours later the animals were perfusion fixed with 4% paraformaldehyde, the L6 spinal cord segments were sectioned and immunoreacted for the Fos protein. Positive cells were counted in 10 sections per segment.

Results

In control bladders ANA concentration was 32.1 pmol/g dry weight. Four hours, 24 hours, three days and 7 days after the onset of inflammation the values rose, respectively, to 45, 42, 59 and 49 pmol/g ($p<0.05$). ANA in concentrations of 1, 5 and 10 μM had no effect on bladder reflex activity. However, 50 and 100 μM ANA increased reflex micturitions/minute from 0.51±0.09 at baselime to 0.73±0.1 ($p<0.05$) and to 0.79±0.09 ($p<0.05$), respectively. CPZ and RTX pre-treatment prevented ANA-induced hyperactivity. SR141716A enhanced the effect of 5 and 10 μM ANA but had no effect on 50 and 100 μM solutions. Bladder instillation of saline induced 51±17 c-fos cells per spinal cord section. ANA increased this number to 80±9

($p<0.05$). Both RTX and CPZ decreased the number of c-fos cells to those found in saline treated animals (57 ± 5 and 51 ± 13, respectively, $p<0.05$).

Conclusions

These results show that ANA increases in the bladder during inflammation and that this endogenous substance may cause pain and enhance reflex micturition through VR1 excitation. The effect of ANA on bladder reflex activity was increased by CB1 receptor blockade. These findings may be relevant to understand pain and bladder overactivity during inflammatory states and may open new therapeutic strategies for the treatment of lower urinary tract symptoms in patients with chronic cystitis.

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