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BLADDER PAIN IN BPS/IC MAY RESULT FROM AN EXAGGERATED RESPONSE OF TRPV1 AND ENHANCED UROTHELIAL ATP RELEASE INDUCED BY CHRONIC ADRENERGIC OVERACTIVITY

Hypothesis / aims of study

Bladder Pain Syndrome/Interstitial Cystitis (BPS/IC) patients present increased activity of sympathetic nervous system. In addition, BPS/IC bladders overexpress TRPV1 and produce increased amounts of ATP [1]. In an animal model of chronic adrenergic stimulation, it was observed that chronic administration of phenylephrine (PHE) induced bladder pain. [2].
Whether this is a mere coincidence or results from a functional cross-talk between the adrenergic system and bladder nociceptive pathways like TRPV1 and ATP are unknown.
In the present work, we postulate that the disproportionate intensity of bladder pain observed during normal bladder filling in BPS/IC patients is the consequence of TRPV1 sensitization and of the enhancement of ATP release from urothelial cells in response to innocuous stimuli caused by chronic adrenergic stimulation.

Study design, materials and methods

To test which adrenoceptor subtype is involved in the PHE induced effect, a group of six female Wistar rats received 2.5 mg PHE/kg, subcutaneously (sc) for 14 days. Another 3 groups of female Wistar rats (n = 6/group), orally received prazosin (a non-specific alpha 1 antagonist), silodosin (selective alpha 1A antagonist), and naftopidil (alpha 1D/1A antagonist) during PHE stimulation. Animals treated with vehicle (saline) were used as controls.
The threshold for mechanical hyperalgesia was evaluated in the lower abdomen before and 14 days after the beginning of PHE treatment, using von Frey filaments. At day 15, animals were anaesthetised and cystometries were performed during saline infusion. We concluded that alpha 1A adrenoceptors were predominantly involved (see results below). Therefore in the following experiments only silodosin was used.

To test whether there is a correlation between adrenoceptors and TRPV1, adult wild type (WT) mice and TRPV1 knockout (KO) mice were sc injected with 2.5 mg PHE/kg, sc for 14 days. Vehicle (saline) injected animals were used as controls. The threshold for mechanical hyperalgesia was evaluated in the lower abdomen before and 14 days after the beginning of PHE treatment, using von Frey filaments. At day 15, animals were anaesthetised and cystometries were performed during saline infusion.

To test if TRPV1 is sensitized during chronic adrenergic stimulation and if this mechanism was alpha 1A adrenoceptors dependent, female Wistar rats received 2.5 mg PHE/kg (with or without silodosin 0.2 mg/kg/day), sc for 14 days. At day 15, animals were anaesthetised and cystometries were performed first during saline infusion, and then during capsaicin infusion. Animals treated with vehicle (saline) were use as control.

To investigate the release of urothelial ATP during adrenergic stimulation, human urothelial cells were grown without and in the presence of PHE (0.62 mM), for 7 days. The effect of mechanical stretch on ATP release was then determined by bioluminescence.
To investigate if the release of urothelial ATP during adrenergic stimulation was mediated by alpha 1A adrenoceptor, human urothelial cells were grown without and in the presence of PHE (0.62 mM) + silodosin (0.5 nM), for 7 days. The effect of mechanical stretch on ATP release was then determined by bioluminescence.

Results

Chronic adrenergic stimulation with PHE decreased mechanical pain threshold of rats from 41.2 +/- 21.0 to 6.7 +/- 2.4 grams ($P<0.001$). Also, PHE treatment increased bladder reflex activity of control rats from 0.44 +/- 0.09 to 1,93 +/- 0.87 bladder contractions/minute ($P<0.001$). Naftopidil treatment did not change the PHE-induced effects, maintaining the pain threshold levels at 6.0 +/- 2.5 ($P<0.001$, compared to control) and bladder reflex activity at 1.9 +/- 0.4 ($P<0.001$, compared to control). However, treatment with Silodosin or with Prazosin reversed PHE induced mechanical hyperalgesia (35.5 +/- 19.4 and 14.2 +/- 7.0, respectively; $P > 0.05$, compared to control) and bladder hyperactivity (0.7 +/- 0.2 and 0.7 +/- 0.2, respectively; $P > 0.05$, compared to control).

Chronic adrenergic stimulation with PHE decreased mechanical pain threshold of wild type mice from 0.04 +/- 0.02 to 0.008 +/- 0.000 grams ($P < 0.05$). Also, PHE treatment increased bladder reflex activity of control rats from 0.47 +/- 0.16 to 1.17 +/- 0.15 bladder contractions/minute ($P < 0.01$). TRPV1 KO mice submitted to PHE treatment did not present any evidence of decreased mechanical pain threshold (0.10 +/ 0.05 to 0.11 +/- 0.06 grams, $P > 0.05$ compared to control TRPV1 KO mice) or increased of bladder reflex activity (0.45 +/- 0.11 to 0.68 +/- 0.15 contractions/minute) $P > 0.05$ compared to control TRPV1 KO mice).

Capsaicin infusion induced an increase in bladder reflex activity of 0.33 +/- 0.15 bladder contractions/minute in control rats. In PHE-treated rats, capsaicin infusion induced an increase in bladder reflex activity of 1.03 +/- 0.30 bladder contractions/minute (P

<0.05). In PHE-treated rat that received silodosin, capsaicin infusion induced an increase in bladder reflex activity of 0.48 +/- 0.05 bladder contractions/minute (P > 0.05, compared to control)

Human urothelial cells release 5.5 +/- 3.2 pmol ATP when subjected to stretch stimuli. Incubation of these cells with PHE increased ATP release upon stretch stimuli to 10.1 +/- 5.5 pmol ATP (P<0.001). Silodosin reduced the increase in ATP release induced by PHE (7.6 +/- 3.7, P<0.001 when compared with PHE treatment).

Interpretation of results

There are two pain mechanisms attributed to BPS/IC patients, one involving the purinergic pathway and another involving TRPV1 receptor. In the present work we show that both this mechanisms can be sensitized/activated through a process mediated by alpha 1A adrenoceptor activation. TRPV1 is an important pain pathway and TRPV1 expression is increased in BPS/IC patients. In addition increased urothelial ATP release is typically observed in BPS/IC patients, which might activate P2X3 nociceptors coursing the bladder suburothelium.

Concluding message

Alpha 1A-mediated adrenergic overstimulation sensitizes TRPV1 and increases ATP release from urothelial cells. Both mechanisms may contribute to the disproportionate pain response to innocuous stimulation seen in BPS/IC patients during innocuous bladder filling.

References

1. Biomed Res Int. 2014;2014:865463. doi: 10.1155/2014/865463.
2. Neurourol Urodyn. 2013 Dec 24. doi: 10.1002/nau.22542.

Disclosures

Funding: Ana Charrua is supported by Fundação para a Ciência e Tecnologia (FCT) fellowship SFRH/BPD/68716/2010. This work was supported by FCT project EXPL/NEU-SCC/0288/2013. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat - Wistar Han
Mice - C57BL/6 **Ethics Committee:** Direção-Geral de Alimentação e Veterinária - Ministério da Agricultura
Ethical committee of Faculty of Medicine of University of Porto