13

Charrua A[1], Cruz C[1], Boudes M[2], De Ridder D[2], Cruz F[3]
1. *Faculty of Medicine of the University and IBMC, University of Porto, Porto, Portugal,* ***2.*** *KU Leuven, Department of surgery, Laboratory of experimental urology, Belgique,* ***3.*** *Faculty of Medicine*

TRPV1 AND TRPV4 ANTAGONISTS HAVE A SYNERGISTIC EFFECT DURING CYSTITIS.

Hypothesis / aims of study

TRPV1 and TRPV4 are known to be expressed in the urinary bladder. TRPV1 is present both in the urothelium and in the urinary bladder nerve fibres. TRPV4 is present in the urothelium, being the two receptors co-expressed in 20% of rat urothelial cells. Recent findings indicate that TRPV4 may also be expressed in bladder afferents. In L6 dorsal root ganglia (DRG) neurons, about 30% of the entire population co-express TRPV1 and TRPV4. However, it is unknown if that neuronal population project to the urinary bladder or if remains constant upon inflammation.

During physiological conditions, TRPV1 does not seem to have a role in the urinary bladder micturition reflex. And although experiments performed with TRPV4 knockout mice indicated that TRPV4 has a role in the control of normal micturition reflex, the application of TRPV4 antagonist into the bladder of control animals did not alter bladder activity. However, during inflammatory conditions, the application of TRPV1 or TRPV4 antagonist has able to reduce of blockade cystitis-induced bladder hyperreflexia.

Therefore, our goals were to study the expression of TRPV1-TRPV4 in L6-S1 DRG population that projects to the urinary bladder, during cystitis, and to study inflamed urinary bladder reflex activity after co-application of TRPV1 and TRPV4 antagonists.

Study design, materials and methods

The urinary bladder of female Wistar rats was injected with fluorogold (FG). Five days after FG injection animals were divided in two groups. Lipopolysaccharide (LPS)-inflamed group was intravesical instilled with 2 mg/ml of LPS, for 1 h. Vehicle-treated group was instilled with saline for 1 h. Twenty-four hours later, animals were perfused and L6-S1 DRG were harvested, sectioned and immunoreacted against TRPV1-TRPV4. Images were acquired and analysed.

In another experimental set, adult female rats were divided in 6 groups. In three groups, bladder inflammation was induced with LPS, 24h prior to experiment. Three groups of vehicle-treated animals were instilled with saline. For experimental procedure, animals were anaesthetised and a catheter was inserted in inferior cava vein for antagonist injection. Then a needle was inserted in the bladder dome and saline was perfused to the bladder, at 6 ml/h, while bladder pressure was being recorded. After 30 minutes of stable cystometry, animals were treated with saline and increasing doses (0.01, 0.1, 1, 10 and 100 □M) of one or two of the antagonists, described as follow: one group of vehicle and one group of LPS treated rats was treated with TRPV1 antagonist SB366791 (SB), one group of vehicle and one group of LPS treated rats was treated with TRPV4 antagonist RN1734 (RN) and one group of vehicle and one group of LPS treated rats was treated with both RN and SB. After cystometry, the urinary bladder of both vehicle and LPS-treated animals were harvested, fixed and Hematoxylin-Eosin stained for histological evaluation.

Results

In vehicle treated animals, L6-S1 DRG showed a large population of FG-positive neurons that were immunoreactive for TRPV1 or for TRPV4. Of these, 36% of TRPV1-IR cells and 31% of TRPV4-IR cells presented TRPV1 and TRPV4 co-localization. In LPS-inflamed animals, L6-S1 DRG also showed a large population of small to medium sized cells that were immunoreactive for TRPV1 or for TRPV4. However, the percentage of cells that co-localize the two receptors drastically decreased upon inflammation.

The urinary bladder reflex activity of vehicle treated animals was unaltered upon treatment with SB, RN or both, at any of the doses tested. LPS-inflamed animals had a higher frequency compared with controls. Treating LPS-inflamed animals with SB or with RN had no statistical significant effect on bladder activity, at all doses. However, when co-applied, the two antagonist reversed LPS-induced hyperflexia at doses of 0.01 □M..

Interpretation of results

The low co-localization of TRPV1 and TRPV4 at physiolocial conditions and a drastic decrease of the receptors co-localization during inflammation further confirm the existence of subtypes of bladder afferents that may have different bladder activity. This observation is in agreement with previous works that describe TRPV4 to be predominantly expressed in capsaicin-resistant fibers. The decrease in the receptors co-localization could be partially explained by the increase in total TRPV1 and TRPV4 expressing cells.

A synergistic activity of TRPV1 and TRPV4 during pathological conditions is here demonstrated for the first time.

Concluding message

Although there a drastic reduction on TRPV1 and TRPV4 co-expression on L6-S1 DRG during inflammation these receptors seem to have synergistic activity. This may be highly relevant for the therapeutic since it is expected to overcome potential side effects of each antagonist, such as TRPV1 antagonist induced-hyperthermia or TRPV4 antagonist-induced urinary retention/overflow incontinence.

Disclosures

Funding: Ana Charrua has a fellowship SFRH/BPD/68716/2010 by Fundação para a Ciência e Tecnologia. **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Rat **Ethics Committee:** Direcção Geral de Veternária