

TRPV1/TRPV4DBL^{-/-} MICE DISPLAY AN INTERESTING URINARY PHENOTYPE IN VIVO.

Hypothesis / aims of study

Both TRPV1 and TRPV4 have been suggested to play a role as mechanosensors in the bladder. TRPV1^{-/-} mice show an altered urinary function, characterised by an increased number of small urinary spots (when voluntary voiding is tested by placing them on filter paper) and an increased bladder capacity during cystometry under urethane anaesthesia.(1) TRPV4^{-/-} mice also have voiding disturbances, characterised by an incontinent-like voiding pattern when placed on filter paper and an increased bladder capacity during conscious cystometry.(2) These experiments suggest that TRPV1 is responsible for setting the micturition threshold under reflex conditions, whereas TRPV4 is responsible for setting the micturition threshold in conscious voiding. Therefore we hypothesized that TRPV1/TRPV4^{Db1^{-/-}} mice will display a totally disturbed urinary phenotype.

Study design, materials and methods

For all experiments male TRPV1^{-/-}, TRPV4^{-/-}, TRPV1/TRPV4^{Db1^{-/-}} and control C57/Bl6 mice, aged 12-15 weeks were used. **Generation of a TRPV1/TRPV4^{Db1^{-/-}} strain:** TRPV1^{-/-} and TRPV4^{-/-} mice strains (both with a C57/Bl6 background) were crossed until TRPV1/TRPV4^{Db1^{-/-}} homozygous breeding pairs were available. **In vivo voluntary voiding behaviour** was tested, by placing the mice for 24 hours on filter paper in rectangular metabolic cages. After 24 hours, the collecting papers were photographed with a digital camera under ultraviolet light. Images were analysed using the Images toolbox of the software MATLAB (R14). **In vivo cystometry:** A small catheter was inserted in the bladder dome and tunnelled to the interscapular region under general anesthesia. After 72h, the catheter was attached to a three-way-tap, connecting it to an infusion pump and a pressure transducer to perform conscious cystometry. **Statistics:** Data are presented as mean ± S.E.M. Data were compared using ANOVA with post hoc Bonferoni test. Significance level p<0,05

Results

TRPV1/TRPV4^{Db1^{-/-}} were obtained by crossing TRPV1^{-/-} and TRPV4^{-/-} mice. The mice bred well and had a survival rate comparable to the wild types.

In vivo voluntary voiding (fig 1): Wild type mice have a characteristic voiding behaviour of voiding predominantly in the corners of the metabolic cages. TRPV1^{-/-} mice show an increased number of small diameter urine spots, represented by a decreased size and increased number of "out of corner" spots. TRPV4^{-/-} mice also have an increased number of "out of corner" spots, but the spot size is not significantly changed. Surprisingly, TRPV1/TRPV4^{Db1^{-/-}} mice show a completely normal voiding pattern, without changes in either spot size or number of "out of corner" spots.

In vivo cystometry (fig 2): When comparing the 4 groups during in vivo conscious cystometry, only the TRPV4^{-/-} mice showed a significantly increased volume threshold for voiding and consequently a lower voiding frequency. TRPV1^{-/-} mice had a tendency to higher volume thresholds, but in the TRPV1/TRPV4^{Db1^{-/-}} mice, these changes were completely abolished. No differences were seen between TRPV1/TRPV4^{Db1^{-/-}} and wild type mice.

Interpretation of results

In this study, we were able to reproduce previously published data about the urinary phenotype of TRPV1^{-/-} and TRPV4^{-/-} mice. We generated a TRPV1/TRPV4^{Db1^{-/-}} mouse strain, but surprisingly, these mice had a completely normalised voiding pattern. These data suggest an interaction between the working mechanisms of TRPV1 and TRPV4. Deletion of one of these channels can cause an imbalance, disturbing normal bladder function. Diseases influencing one of these channels, might be leading to bladder overactivity in a similar way.

Concluding message

TRPV1/TRPV4^{Db1^{-/-}} mice have an interesting normal urinary phenotype. These mice offer new tools to determine the role of TRPV1 and TRPV4 in bladder (patho)physiology.

References

1. Nat. Neurosci. **5**, 856-860 (2002). 2. J. Clin. Invest **117**, 3453-3462 (2007)

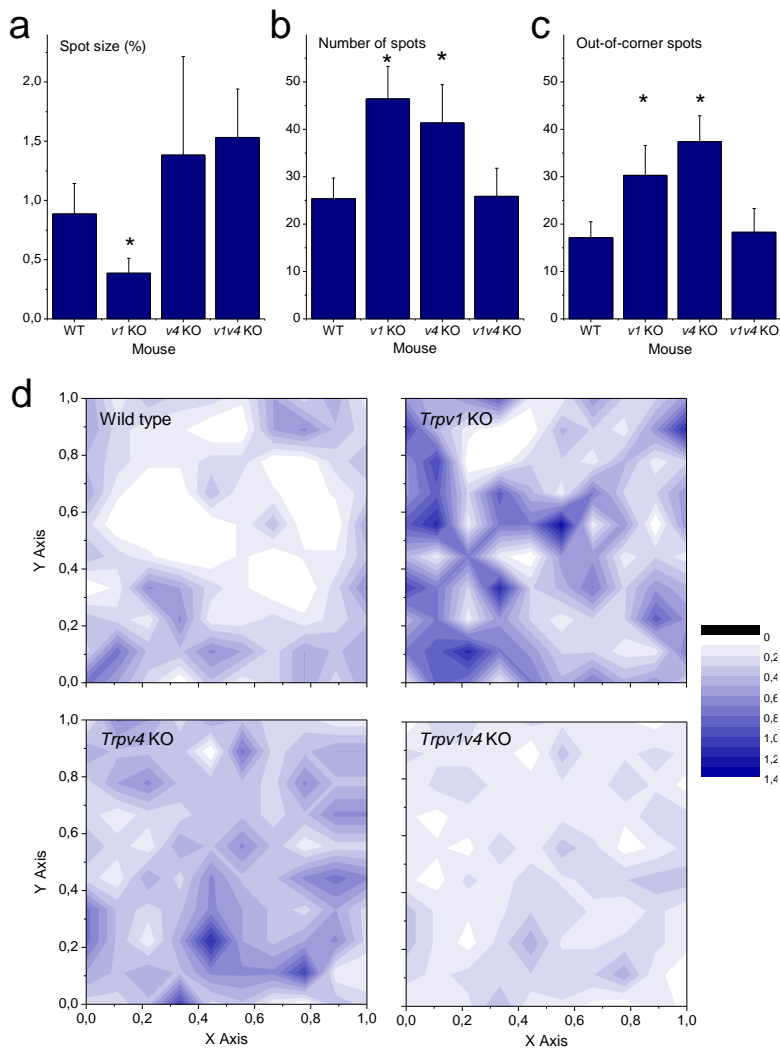


Fig 1. In vivo voiding behaviour in WT (n=15) TRPV1^{-/-} (n=7), TRPV4^{-/-} (n=10) and TRPV1/TRPV4^{Db1-/-} (n=18) mice

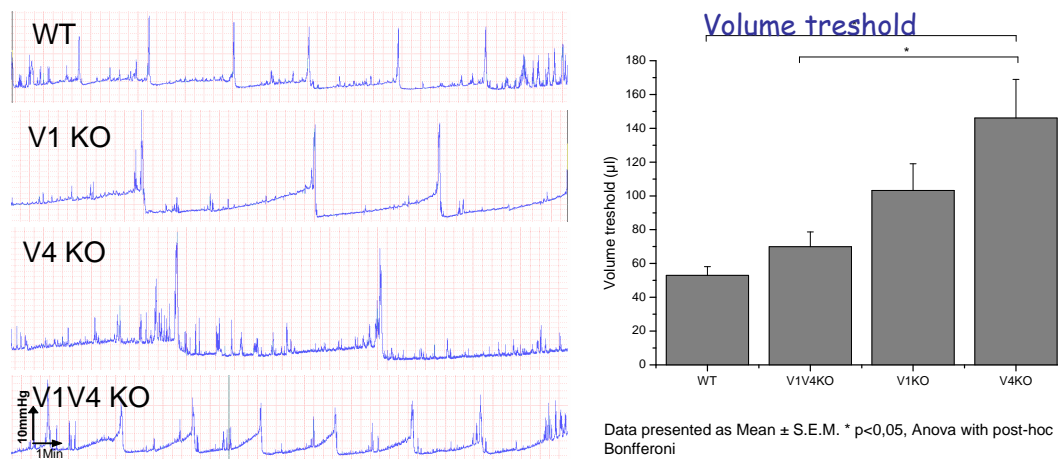


Fig 2. In vivo conscious cystometry. WT (n=9), TRPV1^{-/-} (n=6), TRPV4^{-/-} (n=6), TRPV1/TRPV4^{Db1-/-} (n=9)

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Is this a clinical trial?	No
What were the subjects in the study?	ANIMAL
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	Yes
Name of ethics committee	Ethical Committee For Animal Experimensts (ECD) KULeuven