

APP441-1, A NOVEL TRPV4 AGONIST IS ABLE TO INDUCE BLADDER OVERACTIVITY IN RATS AND MICE.

Hypothesis / aims of study

TRPV4 is a member of the TRP superfamily of cation selective channels. TRPV4 is considered to be a mechanosensitive channel, being gated by osmotic cell swelling and shear stress. TRPV4 is expressed in the urothelium of the bladder, where it functions as a detector of bladder fullness (1). This is supported by the observation that TRPV4^{-/-} mice have hyporeflexic bladders with an increased bladder capacity. The most frequently used agonist for TRPV4, 4αPDD (2) however is unable to induce any effect on the micturition reflex in vivo, probably due to its limited potency. We currently have a new TRPV4 agonist available, called APP441-1. We wanted to test if this agonist is able to activate TRPV4 in urothelial cells and if this agonist can influence the bladder reflex in vivo.

Study design, materials and methods

Animals: 12 week old female Whistar rats and 12 weeks old male wild type and TRPV4^{-/-} mice with a C57BL/6J background were used for in vivo experiments. All animal experiments were carried out in accordance with the European Union Community Council guidelines and were approved by the local ethics committee.

Ca²⁺ imaging on cultured urothelial cells: Primary urothelial cells cultures were prepared as described by others. Urothelial cells were collected from WT and TRPV4^{-/-} mouse bladders. 12-24h after isolation, intracellular Ca²⁺ recordings were performed using Fura2 as a Ca²⁺ indicator.

In vivo cystometry:

Cystometry in rats: Cystometry was performed in conscious rats. Intravesical pressure recordings are performed during the instillation of saline at 200μL/min. Baseline recordings were performed for 30min, followed by the infusion of 100μM APP441-1 in saline for 30min.

Cystometry in mice: Cystometry in mice was performed as previously described (1). Cystometry was performed under urethane anesthesia (1.2mg/kg), with an infusion rate of 20μL/min. Baseline recordings were performed for 30min, followed by infusion of 100μM APP441-1 in saline for 30 min.

Results

Ca²⁺ imaging on cultured urothelial cells: Application of 1μM APP441-1 induced an increase of [Ca²⁺]_{in} in 91% (179/195) of the cells from wild-type but not in cell from TRPV4^{-/-} mice.

In vivo cystometry:

In conscious rats (n=4) application of 100μM APP441-1 induced a significant increase in voiding pressure (from 59.1 ± 3.5 to 91.3 ± 8.1 mmHg, p<0.05) and decrease in the intercontractile interval (ICI) to 65% of the baseline value (from 270 ± 27 to 176 ± 27sec, p<0.05).

In urethane anesthetised mice, application of 100μM APP441-1 induced bladder overactivity, characterised by a 53% decrease in the ICI (from 169.4 ± 31.3 to 12.6 ± 25.2sec p<0.05). In comparison to wild types, TRPV4^{-/-} mice had a lower baseline voiding frequency. Moreover, application of 100μM APP441-1 did not induce any effects in these TRPV4^{-/-} mice.

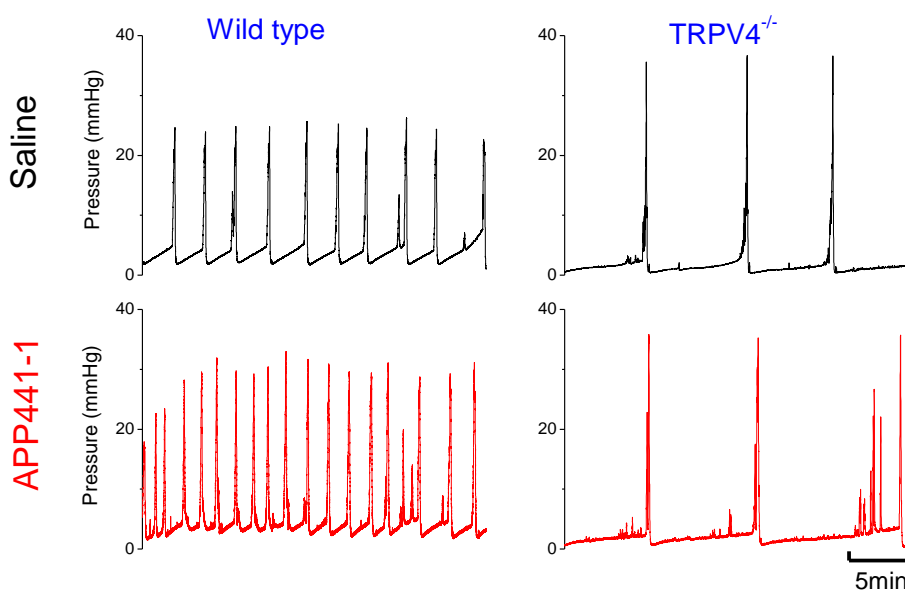


Fig. 1 *In vivo* cystometry in wild type and TRPV4^{-/-} mice, during infusion of saline or 100μM APP441-1 in saline

Interpretation of results

Using Ca^{2+} imaging, we showed that $1\mu\text{M}$ APP441-1 induces a Ca^{2+} influx in urothelial cells derived from wild type, but not from TRPV4^{-/-} mice, indicating that APP441-1 is able to selectively activate TRPV4 in urothelial cells.

In conscious rats, APP441-1 induced bladder overactivity, characterised by an increase in voiding pressure and voiding frequency. Also in urethane anaesthetised mice, APP441-1 increased the voiding frequency in wild type, but not in TRPV4^{-/-} mice. These data indicate that activation of TRPV4 can induce bladder overactivity in both awake and urethane anaesthetised animals.

Concluding message

APP441-1 is a specific agonist for TRPV4 in vitro and in vivo. In vivo activation of TRPV4 can induce bladder overactivity, indicating that TRPV4 is an important target for the treatment of sensory bladder dysfunction.

References

1. J Clin Invest 2007;117(11):3453-62
2. J Biol Chem 2002;277(16):13569-77

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<i>Is this a clinical trial?</i>	No
<i>What were the subjects in the study?</i>	ANIMAL
<i>Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?</i>	Yes
<i>Name of ethics committee</i>	Animal Ethics Committee of KU Leuven