

THE VITAMIN D ANALOGUE BXL-628 ALTERS EXCITATION CONTRACTION COUPLING IN THE ISOLATED BLADDER IN AGED WILDTYPE MICE DIFFERENTLY COMPARED TO BLADDERS FROM AGE-MATCHED TRANSGENIC ALZHEIMER'S DISEASE MICE

Hypothesis / aims of study

This is the first study to provide insights into the mechanisms involved in dysregulation of the urinary bladder in a transgenic model of Alzheimer's disease (AD). AD is the most common neurodegenerative disorder and severely deteriorates patients' quality of life. One of the most debilitating autonomic dysfunctions related to AD is urinary incontinence. Transgenic AD mice show besides behavioural characteristics of Alzheimer's disease, altered micturition behaviour but also morphological changes within the bladder (1). In addition, altered contractile responses to KCl and muscarinic stimulation have been identified in an *ex vivo* setting (2). To identify novel potential treatment targets for patients suffering from AD and bladder dysfunctions, more mechanistic insights into the pathophysiology of bladder dysfunctions in AD patients are needed. Systemically, Vitamin D acts as an antioxidant and anti-inflammatory agent. On a bladder level, its inhibition of the RhoA/Rho kinase signalling pathway, reduction of calcium sensitisation leads to a higher threshold of muscarinic receptor stimulation. We therefore hypothesize that incubation with a Vitamin D3 analogue counteracts a potential misbalance of signalling systems within the dysfunctional AD.

Study design, materials and methods

Bladders and the proximal urethra from ten mice of the transgenic Alzheimer's disease model APPswe/PS1ΔE9 and 10 wildtype (WT), aged 91-93 weeks, were dissected, transurethrally catheterised and transferred to a heated organ bath (20 mL, 37 °C) containing continuously aerated Krebs' solution. Subsequently, the bladder was filled to 90 μl with a filling rate of 0.2 ml/hour. After a resting period of 30 minutes, washing steps were performed, followed by a sequence of stimulations. Bladders were stimulated with electrical field stimulation (EFS) with muscle specific parameters (5 ms pulse duration, 6V, 8Hz), EFS with nerve specific parameters (24 V, 80 Hz), carbachol (1 μM) and KCl (100 mM) stimulation. Carbachol and KCl stimulations were terminated by washing steps. After a first sequence of all four stimulations, bladders were incubated for 10 minutes with the vitamin D3 analogue Bxl-628. During incubation with Bxl-628 a second sequence of stimulations, identical to the first sequence was given.

Results

Bladders originating from transgenic AD mice behaved differently to stimulation with KCl and EFS with nerve parameters compared to WT bladders: In bladders originating from transgenic AD mice, a decreased amplitude was recorded in response to KCl stimulation compared to WT mice. In addition, the time that was needed to reach the maximum of the initial peak of EFS evoked nerve stimulation was increased in AD mice compared to WT mice, after incubation with Bxl-628.

Effects of incubation with the vitamin D3 analogue Bxl-628 were detected in both groups. However, these effects were not consistent between bladders originating from AD and WT mice. Incubation with the vitamin D3 analogue resulted in an increased amplitude of the KCl response in bladders of WT mice (Fig. 1). Furthermore, the time interval that was needed to reach the initial peak of the contractile responses was decreased after incubation with Bxl-628 for the KCl response in WT bladders. The EFS response with muscle specific parameters in both, WT and AD bladders, and the EFS response with nerve specific parameters in WT bladders were also decreased ($p=0.027$, $p=0.019$ and $p=0.002$, respectively). The time interval needed to reach the initial response was significantly increased after Bxl-628 and carbachol stimulation in AD bladders ($p=0.048$).

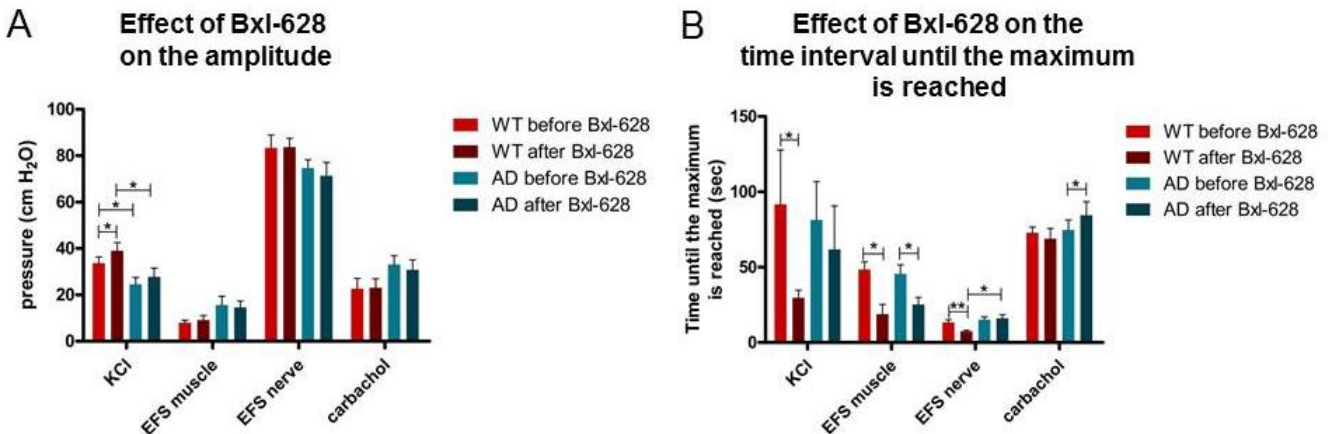


Figure 1. Effect of the Vitamin D3 analogue Bxl-628 on amplitude and time interval until the maximum is reached of KCl stimulation, electric field stimulation of muscles and nerves, as well as carbachol stimulation in transgenic Alzheimer's disease and wildtype mice. In panel A, the effect on the amplitude of the different contractions is depicted for both, wildtype (WT) mice and transgenic Alzheimer's disease (AD) mice. In transgenic AD mice the amplitude of a KCl evoked contraction was significantly decreased compared to wildtype (WT) mice before and after vitamin D receptor activation. In WT mice, incubation with Bxl-628 resulted in an increased amplitude of the KCl response. In bladders of transgenic AD mice, no significantly effect on the amplitude was detected. The effect on the time interval which is needed to reach the maximum of the initial peak is shown in panel B. After Bxl-628 incubation, an increased time interval was measured in transgenic AD bladders compared to bladders originating from WT mice. Incubation with Bxl-628, resulted in a decreased time interval which was needed to reach the maximum of the initial peak after KCl stimulation and EFS stimulation using nerve specific parameters in WT bladders. Regarding electric field stimulation (EFS) using muscle-specific parameters, Bxl-628 incubation decreased the time interval significantly in both groups, WT and AD bladders. In bladders originating from transgenic AD mice, the response to carbachol was increased after Bxl-628 incubation.

Interpretation of results

In control isolated bladder preparations, effects detected after the Vitamin D analogue Bxl-628 altered responses to excitatory pharmacological stimulation by increasing the amplitude of KCL stimulation and decreasing the time interval which was needed to reach the initial maximum of contractile responses to KCl, EFS muscle and nerve stimulation. However, in bladders of transgenic AD mice, the amplitudes after Bxl-628 did not change significantly and the time interval which was needed to reach the maximum of the initial peak was increased after carbachol stimulation but decreased after EFS muscle stimulation. Vitamin D3 exerts its effects by interaction with several signalling systems. Muscarinic activation by carbachol includes activation of L-type calcium channels to induce contraction, as well as Rho kinase activation. The incubation with Bxl-628 and stimulation with different pharmacological agents may help to identify the changes in excitation coupling in AD. A counteracting effect in AD compared to WT bladders might even indicate a possible role of this agent in correcting a misbalance in excitation contraction coupling in AD bladders.

Concluding message

We identified a couple of signalling systems which might be of importance in bladder excitation contraction coupling associated with AD, such as the Rho A/Rho kinase system and calcium signalling in general. Future research should focus on identifying the role of potential amyloid-beta aggregation within the bladder wall to elucidate which interaction with specific proteins or ion-channels takes place, thereby influencing bladder function.

References

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Disclosures

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