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CALCIUM SIGNALLING IN THE ISOLATED URINARY BLADDER IS NOT ALTERED IN A TRANSGENIC ALZHEIMER'S MOUSE MODEL

Hypothesis / aims of study

Alzheimer's disease (AD) is a progressive, neurodegenerative disease, which is characterized by memory loss. Compared to 13% of age-matched controls, 53% of patients suffering from dementia show urinary incontinence. Urinary incontinence is defined by the International Continence Society as ''involuntary loss of urine that is objectively demonstrable and presents a social or hygiene problem' and is one of the main reasons for institutionalization. It has frequently been hypothesized that prefrontal dysfunction in Alzheimer's disease causes incontinence (1). However, urinary incontinence can occur early in the course of disease and it has been hypothesized that morphological and cellular alterations in the bladder take place. The precise modifications and the impact on bladder function are yet to be discovered. Unveiling these alterations can have implications for the treatment of urinary incontinence in dementia-related disorders and might therefore improve the quality of life of patients suffering from AD. Previously, we have investigated vitamin D signalling, which has been shown to be altered in AD related pathology when using the vitamin D analogue BXL-628. Application of BXL-628 has been shown to modify physiological excitation-contraction coupling. Moreover, vitamin D receptor activation has been proven to upregulate L-type calcium channels in the bladder (2). We therefore, hypothesized that changes in excitation-contraction coupling in AD are caused by alterations in calcium signalling.

Study design, materials and method

In this study, we used the *post-mortem* isolated bladders of 40-week-old, female transgenic mice. The mice were bred on a C57BL/6 background possessing the apolipoprotein E3 (APOE3) gene and a (human) mutation for presenilin and amyloid precursor protein (APP; n=9) or were wildtype (WT; n=6) controls, showing no genetic alterations.
The bladders were catheterised and placed into a heated organ bath (20 ml, 37°C) consisting of aerated Krebs solution (pH 7.4). The bladders were then filled with 90µl Krebs solution at a filling rate of 0.2 ml/h, followed by a 30 min resting period. After subsequent washing steps, the bladders underwent a series of electrical stimulations in the presence of increasing concentrations of verapamil (10nM, 100nM, 1µM, 10µM). The bladders were stimulated using electrical field stimulation (EFS) with muscle specific parameters (50ms pulse duration, 6V, 8Hz) and nerve specific parameters (1ms pulse duration, 24V, 80Hz) for 1 min durations with a 2 min resting period after each applied parameter. Initial stimulation occurred in the absence of verapamil, succeeded by 10 min incubation periods following the addition of increasing concentrations of verapamil.

The maximum pressure amplitude and the time to reach the maximum amplitude were calculated offline and compared by means of a Mann-Whitney t-test in the absence of verapamil and by one-way ANOVA test in the presence of verapamil between bladders of WT and AD animals for muscle and nerve parameters separately.

Results

There were no statistically significant differences between bladders in the absence of verapamil from WT and AD mice regarding the maximum amplitude (p=0.48) and the time taken to reach the maximum amplitude (p=0.27) upon muscle stimulation, and the maximum amplitude (p=0.57) and the time taken to reach the maximum amplitude (p=0.22) upon nerve stimulation.

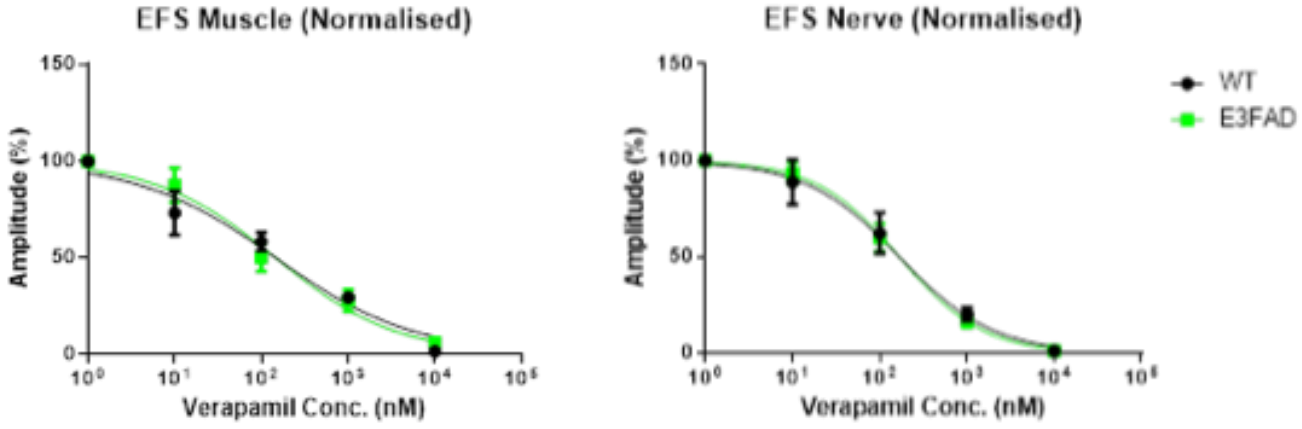

Figure 1. Maximum amplitude upon muscle (left) and nerve (right) EFS in the presence of increasing concentrations of verapamil in bladders of wildtype (black) and transgenic (green) animals.

The addition of verapamil to both bladders from WT and AD animals decreased the normalized amplitude during nerve and bladder stimulation in a dose-dependent manner. Yet, no statistical differences were observed between EFS in the presence of increasing verapamil concentrations between WT and AD animals when applying muscle and nerve specific parameters.

Interpretation of results

For isolated urinary bladder preparations, there were no statistically significant differences detected upon application of EFS before and after exposure to the L-type calcium channel blocker verapamil. Nearly identical patterns of bladder function were observed during EFS when applying both muscle and nerve specific parameters between AD and wild type mice.

Concluding message

The goal of this study was to investigate the role of L-type calcium channels in AD related urinary pathology. We concluded that bladders obtained from animals with a transgenic background, which resembles the human phenotype of AD, do not appear to show alterations in their L-type calcium channel mediated signalling upon exposure to verapamil.

Considering that it is not known what the role of the central nervous system and the influence of peripheral alterations in AD is, this setup of using isolated *ex vivo* bladders facilitated the assessment of potential peripheral and pharmacological targets. The fact that no differences were found between AD and WT bladders before and after exposure to a calcium channel blocker pleads for a role of other signalling cascades downstream of the vitamin D receptor, such as the RhoA/Rho kinase system causing modifications in excitation contraction coupling in AD. Besides, this study emphasizes the necessity to further investigate central and peripheral pathways of signal transduction in AD specifically related to urinary storage dysfunction.

References

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Disclosures

Funding: None **Clinical Trial:** No **Subjects:** ANIMAL **Species:** Mice **Ethics Committee:** Dierethische commissie Maastricht University