

THX-B, AN ANTAGONIST TO RECEPTOR P75^{NTR} IMPROVES BLADDER PARAMETERS IN AGING MICE

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Abstract ID: 24538

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INTRODUCTION

Nerve Growth Factor (NGF) regulates the development and survival of neurons. Its levels in urine are decreased in elderly people diagnosed with overactive bladder (OAB) and were linked to high activity of enzyme MMP-9, the protease that degrades NGF. Animal models of diabetic bladder dysfunction present the same characteristics. Bladder parameters in type 1 diabetic mice and urinary NGF are improved by chronic treatment with THX-B, an antagonist of the proinflammatory receptor p75^{NTR}.

OBJECTIVES

We here aim to determine the functional benefit of p75^{NTR} antagonism on the bladder in aging mice.

METHODS/MATERIALS

Male and female C57BL/6J mice aged 6- and 12-months were injected once weekly for four weeks with either PBS (control) or THX-B. Voiding spot test (VSA) was used to measure urination behaviors and patterns, including total urine volume, frequency of urination, and volume per micturition. Organ baths were used to assess bladder contractility with regards to different stimuli including KCl, Electrical Field Stimulation and Carbachol. NGF and proNGF levels in collected urine were measured using ELISA kits. MMP-9, was semi-quantified by immunoblotting.

MAIN FINDINGS

Compared to controls treated with PBS, voiding behavior and bladder contractility were improved in the 12-month-old mice after chronic treatment with THX-B.

RESULT 1

Voiding spot Assay (VSA): Total urine volume and volume per micturition were reduced in both 12 months male and female mice.

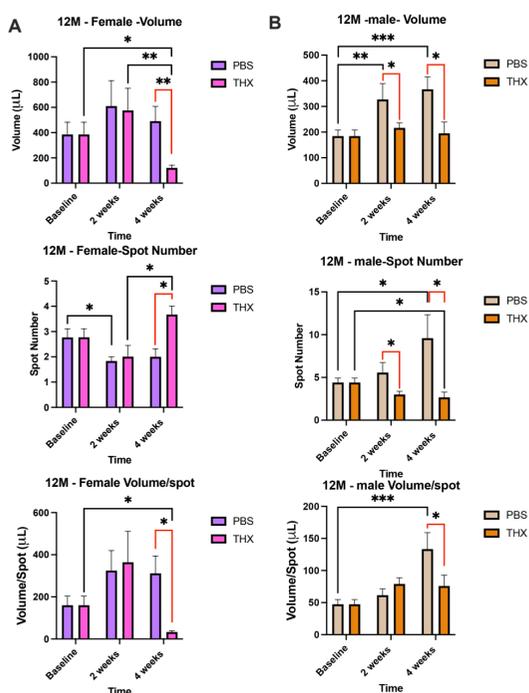


Figure 1. Voiding Spot Assay (VSA) for (A) female and (B) male mice measuring total urine volume (µL), voiding frequency and volume per micturition over a 4 hour period at baseline, before treatment and at 2-weeks and 4 weeks of treatment. N=5-8 mice in each group at each time point. All comparisons were done at a particular time point using two-way ANOVA with post-hoc Tukey to compare time (*P<0.05), (**P<0.005), and (***)P<0.001, and Sidak to compare treatment (*P<0.05) in red.

RESULT 2

Ex vivo, bladder contractility stimulated by KCl (60 mM), Electrical Field Stimulation (16 Hz) and Carbachol was reduced in strips from male mice but increased in strips from female mice after THX-B treatment.

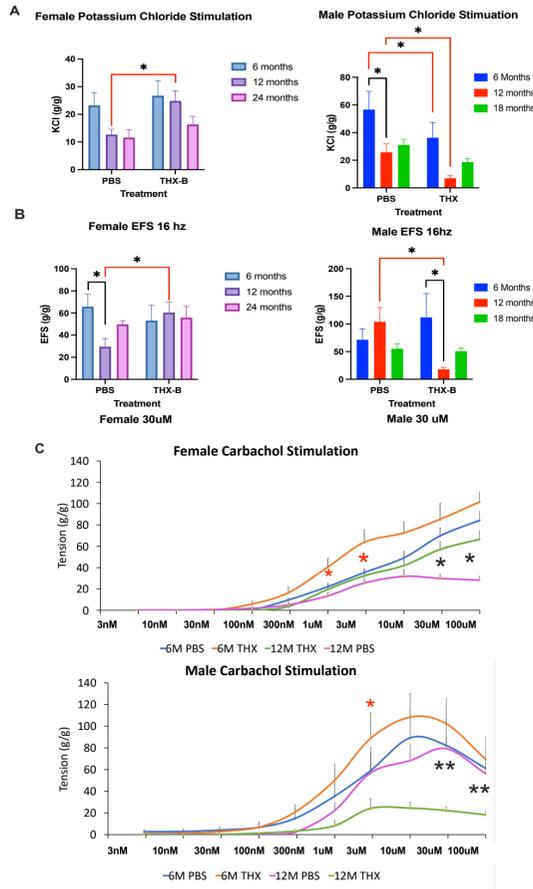


Figure 2. Bladder strips from female and male mean response to (A) potassium chloride (KCl) (60 mmol/L), (B) Electrical Field Stimulation at 16Hz and (C) Carbachol at 30 µM. Strips derived from 6-month, 12-month and 18 or 24-month-old mice treated with THX-B or PBS control. (n=9-11). All comparisons were done at a particular time point using two-way ANOVA with post-hoc Tukey to compare age (*P<0.05), (**P<0.005), and (***)P<0.001, and Sidak to compare treatment (*P<0.05) in red.

RESULT 4

THX-B increased NGF urine levels in male and female 12-month-old mice while MMP-9 activity was decreased only in female mice.

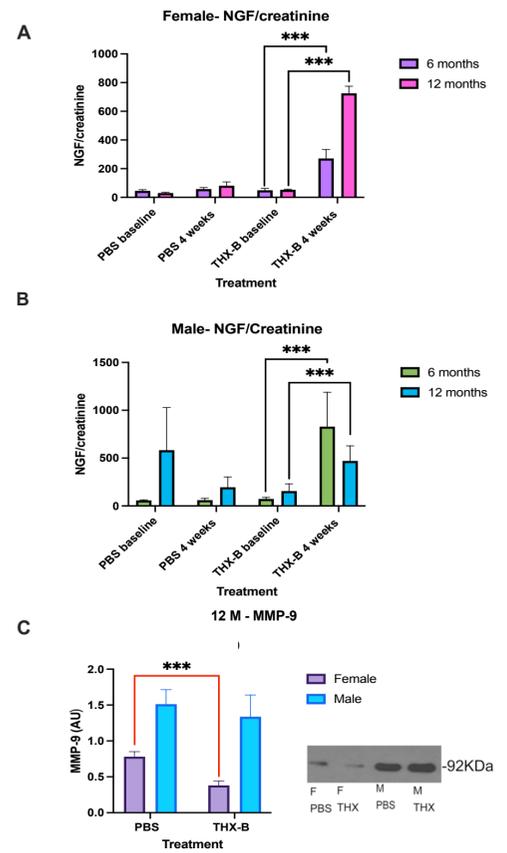


Figure 4. (A) Female (B) Male NGF levels in urine of mice aged 6 and 12 months. Proteins levels were measured using a specific Elisa Kit. ***p<0.001 using a student t-test, n=4 in each. (C) Western blot analysis of MMP-9, from bladder tissue extracts of 6 month and 12 months female and male mice treated with PBS or THX-B. ***p<0.001 PBS vs THX-B; all comparisons were done at a particular time point using student t-test. N=6 for each group.

RESULT 3

Conscious cystometry results revealed a decrease in maximal voiding pressure, basal pressure, spontaneous activity, and micturition volume in female mice treated with THX-B compared to controls. In males, THX-B appears to decrease the maximal voiding pressure as well as the residual volume compared to controls.

A

Female-12 months	PBS	THX-B
Maximal Pressure (cmH2O)	103.3005333	* 44.85767778
Threshold Pressure (cmH2O)		51.673
Basal Pressure (cmH2O)	41.48108	* 15.01640833
Intermicturition Pressure (cmH2O)	57.20465	* 23.48618333
Spontaneous Activity (cmH2O)	17.41065	* 8.469775
Intercontraction interval (s)	389.25	* 172.4
Bladder Capacity (mL)	0.14705	0.067842593
Micturition volume (mL)	0.0749375	* 0.029616667
Residual volume (mL)	0.0871	0.038225926
Bladder compliance (mL/cmH2O)	-0.00407904	-0.003413547

B

Male-12 Month	PBS	THX-B
Maximal Pressure (cmH2O)	100.40075	* ↓ 65.86575
Threshold Pressure (cmH2O)	50.45666667	45.07
Basal Pressure (cmH2O)	27.51	24.776
Intermicturition Pressure (cmH2O)	40.485	37.01
Spontaneous Activity (cmH2O)	12.975	12.234
Intercontraction interval (s)	473.875	297.664
Bladder Capacity (mL)	0.223774306	0.140563556
Micturition volume (mL)	0.0356775	0.049736
Residual volume (mL)	0.235233426	* ↓ 0.090827556
Bladder compliance (mL/cmH2O)	0.006087498	0.0084134

Figure 3. Conscious cystometry after Bladder catheter implantation on 12 months (A) female and (B) male aging mice. n = 6-7 in each group. *p < 0.05 control vs THX-B using student-t-test.

CONCLUSION

These findings suggest that THX-B has an age-specific efficiency, involving enhanced NGF expression through decrease in MMP-9, at least in female mice. Our findings suggest that THX-B might be used as a therapeutic tool to improve OAB.



The authors have no potential conflict of interest.