213

NEUROGENIC BLADDER CAUSES MARKED BLADDER REMODELING AND ALTERED PAIN RESPONSE IN MICE WITH EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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INTRODUCTION AND OBJECTIVES: Multiple sclerosis (MS) is an inflammatory disease of the central nervous system which causes neurogenic bladder (NGB). Experimental autoimmune encephalomyelitis (EAE) mice have been widely used in MS research as they demonstrate similar T cell mediated demyelination and neurodegeneration. We have previously reported the evidence of NGB in EAE mice. The aim of this study was to examine the stages of neurologic deficit in relation to bladder remodeling, and examine the EAE mice responses to visceral pain.

METHODS: Female SJL mice (n=118) were immunized with mycobacteria tuberculosis along with Freund's adjuvant (control), and then of this sample, 100 mice were injected with myelin protolipid protein (PLP 139–151) to induce EAE. On days 0, 3, and 7 all mice received intraperitoneal injection of purified Bordetella pertussis toxin. Daily weights, and neurologic clinical scores (CS) were assessed for signs of neurodegeneration and graded as 1–5. Then, the animals were divided into two groups. The first group (n=42) was euthanized at 70 days, bladders were harvested and weighed, and then prepared with routine hematoxylin-eosin and Masson's trichrome staining. Digital imaging analysis was used to quantify bladder cross-sectional areas, and smooth muscle, urothelium, and collagen (Image-Pro Plus). The second group (n=76) was assessed after short and long term (day 70) for signs of neurodegeneration (CS 1–5), along with visceral pain responses from perpendicular pressure with calibrated von Frey hair monofilaments to the hind paw and supra-pubic region.

RESULTS: Significant increases in selective bladder areas of control compared to EAE (CS4) for total wall, urothelium, smooth muscle, collagen, and inner lumen (2.31 vs. 3.50, P=0.014; 0.3078 vs. 0.62, P=0.001; 1.49 vs. 2.00, P=0.036; 0.50 vs. 0.87, P=0.021; 0.22 vs. 0.71, P=0.001, respectively) were seen. Also noted were statistically significant increases from the outer perimeter (P=0.004), and percent of urothelium and collagen (P=0.0008 and P=0.004, respectively). Along with CS increase , the threshold response (>50%) to pain stimuli dramatically increased and visceral pain response decreased with short term supra-pubic area of 2.83g vs. 3.84g; and hind paw 3.61g vs. >4.56g (control vs. CS3, respectively).

CONCLUSIONS: EAE causes neurologic deficit in mice and contributes to marked bladder remodeling and a dramatic decrease in visceral pain response, which proportionally worsens as the neurodegeneration progresses. The EAE mouse could be used as a robust tool for studies of NGB.

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214

URINARY BLADDER DYSFUNCTION IN MICE NULL FOR ECTONUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE 1 AND 5'-NUCLEOTIDASE

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INTRODUCTION AND OBJECTIVES: Purinergic signaling via extracellular nucleotides/nucleosides plays a crucial role in normal urinary bladder function and appears dysregulated in diseases like diabetes and interstitial cystitis. The equilibrative balance in the extracellular levels of nucleotides and nucleosides is maintained by ectonucleoside triphosphate diphosphohydrolase enzymes of the CD39/ENTPD family, CD73/5'-nucleotidase (5'-NT) and alkaline phosphatases. We undertook to define the expression of these ecto-ATPases and the functional consequences of genetically ablating ENTPD1 and 5'-NT in mice.

METHODS: RT-PCR, Western blot, and immunofluorescence (IF) were used to define the expression and distribution of nucleotidases. Morphological and functional evaluation of ENTPD1−/− and 5'-NT−/− mice were assessed by H&E staining, in cage micturition patterns and cystometrograms (CMG).

RESULTS: ENTPDs 1, 2, 3, 8 and 5'-NT in bladder were shown at the mRNA and protein level. IF revealed both ENTPD1 and 5'-NT on cell membranes of smooth muscle cells. Micturition patterns indicated that ENTPD1−/− mice were incontinent with random urine leakage. CMGs showed a striking 2–4 fold increase in intercontractile interval (ICI). Bladders of ENTPD1−/− mice were 60% heavier and appeared dilated (P<0.05). H&E staining confirmed bladder dilatation, with fragmented muscle bundles. In contrast, 5'-NT−/− mice were also incontinent and exhibited irregular ICI with an increase in detrusor contractile activity, a phenotype resembling overactive bladder. Bladders of 5'-NT−/− mice were 100% heavier (P<0.05); H&E staining indicated bladder enlargement. Immunoblotting showed that P2X1 was significantly downregulated (55% of controls, P<0.01), and 5'-NT was significantly upregulated (1.7 fold, P<0.05) in bladders from ENTPD1−/− mice; features consistent with elevated ATP levels. Both ENTPD1 and adenosine receptor, A2b were significantly upregulated in 5'-NT−/− mice (1.3 fold, P<0.01 and 1.6 fold, P<0.05 respectively), presumably due to decreased adenosine production.

CONCLUSIONS: The data indicate an important role for ectonucleotidases in normal bladder function. As ENTPD1 and 5'-NT both localize to detrusor, the loss of either results in compensatory changes in the other, suggesting regulatory feedback. Appropriate regulation of ATP and adenosine levels is important in smooth muscle relaxation and contraction via P2X1 and A2b receptor signaling.

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215

TREATMENT WITH OXYBUTYNIN CHLORIDE IS NOT EFFECTIVE IN DECREASING THE INCIDENCE AND SEVERITY OF AUTONOMIC DYSREFLEXIA IN PERSONS WITH SCI

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INTRODUCTION AND OBJECTIVES: Oxybutynin chloride is currently used for the treatment of detrusor hyperreflexia in persons with SCI, a spastic condition of the bladder that often triggers autonomic dysreflexia (AD). AD is a sympathetically mediated acute elevation in systemic blood pressure triggered by a stimulus below the neurological level of injury. Oxybutynin has a high affinity for muscarinic receptors located in the bladder and effectively blocks carbachol-induced contractions; it is assumed that this action decreases the afferent response from the bladder, thus lessoning the incidence and severity of AD. The study objective is to compare the changes in SBP during clinical urodynamics in 12 individuals with SCI chronically treated with oxybutynin and in 11 persons with SCI without treatment.

METHODS: 23 persons with chronic SCI (>1 yr) above T6 exhibiting detrusor hyperreflexia underwent routine urodynamics with fill and void cystometry during which beat-to-beat BP was monitored and recorded from a finger arteriolar. Unpaired t-tests were performed on the percent change of systolic blood pressure (SBP) from baseline to maximum cystometric capacity (MCC) between groups.

DESIGN: Cross sectional, prospective, observational.

RESULTS: Comparing the treated to the untreated group, the incidence (# of episodes/study group, respectively) and severity (average rise in BP/ study group, respectively) of AD was comparable. Although the increase in SBP was higher in the treated (22.47%) compared to the untreated group (9.49%), albeit not significantly (p=0.11), MCC and detrusor pressure were also marginally increased (458 vs. 439 cc and 39 vs. 18.0 cmH20; respectively).

CONCLUSIONS: These preliminary findings suggest that while treatment with oxybutynin decreases uninhibited detruser contractions and increases bladder capacity, oxybutynin did not prevent the development of AD during bladder filling. We propose that the inhibitory