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Putting the past behind us: Social stress-induced urinary retention can be overcome

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Summary

Introduction
To study the pathophysiology of dysfunctional voiding, we have previously developed a model of stress-induced voiding dysfunction. We have shown that cyclosporine A (CsA), an inhibitor of the Ca^{2+}–calmodulin complex, can prevent social stress-induced urinary retention. However, treatment with cyclosporine has not had an effect on the increase in the stress peptide corticotrophin-releasing factor (CRF) in Barrington's nucleus, which is involved in the micturition pathway.

Objective
We now investigate whether cyclosporine administered after stress can reverse the abnormal voiding phenotype, and whether it has effects on the bladder wall itself, or on the stress response within Barrington's nucleus.

Materials and methods
Six-week old Swiss–Webster mice were exposed to aggressor males for 1 h a day, followed by 23 h of barrier separation. In a long-term trial, 1 month of stress was followed by single-cage housing for 6 months. In a separate CsA reversal trial, mice either received CsA in drinking water or had plain drinking water during 1 month of single-cage housing during recovery. Bladder contractile function was examined on a Guth myograph. Nuclear translocation of myocyte enhancing factor (MEF)-2 and NFAT (nuclear factor of activated T cells) in the bladder was assessed using electrophoretic mobility shift assays (EMSAs). The expression of CRF was determined in Barrington's nucleus using *in situ* hybridization.

Results
Voiding dysfunction persisted for up to 6 months after stress exposure while mice recovered in single-cage housing. In the CsA reversal trial, voiding patterns improved when they received CsA in water during single-cage housing following stress, whereas those that underwent single-cage housing alone had persistent abnormal voiding (Fig. A). There was no difference between CRF levels in Barrington's nucleus between reversal groups ($p = 0.42$) (Fig. B), possibly indicating a direct effect on the bladder rather than a persistent stress effect. There were no differences in the contractility of bladder wall muscle. CsA decreased the nuclear translocation of MEF-2 and NFAT induced by stress (Fig. C,D).

Conclusion
CsA reverses stress-induced urinary retention, but does not change the stress-induced CRF increase in Barrington's nucleus. Furthermore, bladder smooth muscle contractility is unchanged by CsA; however, there are changes in the levels of the downstream transcription factors MEF-2 and NFAT. We suspect that additional CsA responsive neural changes play a pivotal role in the abnormal voiding phenotype following social stress.

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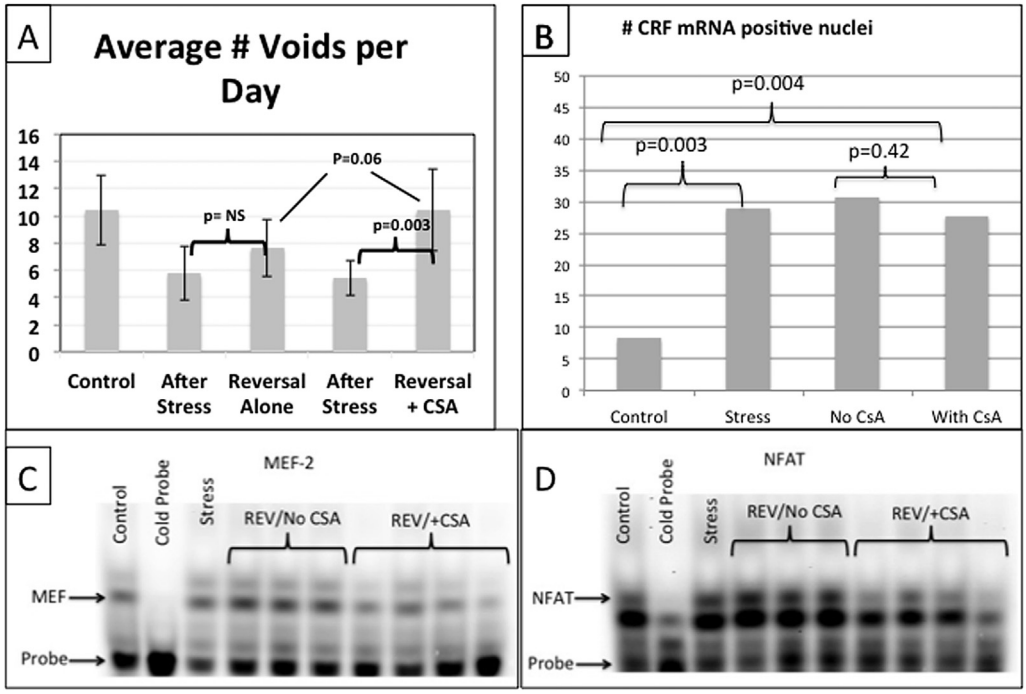

Figure (A) Average number of voids in 10-week-old control Swiss—Webster male mice, compared with number of voids after 1 month of stress, and after stress followed by 1 month of single-cage housing alone or 1 month of single-cage housing with CsA administration. (B) There was no significant difference between stress and recovery groups on fluorescent in situ hybridization analysis; however, all were significantly increased from control (C,D) Electrophorectic mobility shift assay (EMSA) performed for quantification of nuclear protein levels of myocyte enhancing factor 2 (MEF-2) and nuclear factor of activated T cells (NFAT) using infrared-labeled DNA probes.

Introduction

Dysfunctional voiding remains a common reason for pediatric urologic consultation. Patients present with a range of symptoms from frequent urinary tract infections to urinary urgency and incontinence due to incomplete bladder emptying. Voiding dysfunction can be idiopathic, or may develop after painful elimination (due to trauma, infection, or even constipation). Many patients are treated with behavioral modification, however, with increased water intake, timed voiding, and treatment of underlying constipation. However, for those whose symptoms fail to improve, there are few non-invasive treatments and pharmacologic options beyond anti-cholinergic medications.

We have previously developed and studied a model of stress-induced voiding dysfunction in order to study the pathophysiology of stress-induced urinary retention, which mimics some presentations of voiding dysfunction [1,2] The calcineurin—NFAT (nuclear factor of activated T cells) pathway was selected as a possible factor in the long-term effects of voiding dysfunction based upon early work on the modulation of bladder fibrosis and hypertrophy by calcium as a response to bladder outlet obstruction [3]. Later, Crabtree and Olson [4] found that calcium upregulated calcineurin, which in turn altered gene expression by dephosphorylation of NFAT (a downstream transcription factor of calcineurin). This led to the association of social stress voiding dysfunction and calcineurin. Based upon early investigations into the effect of the brain on bladder function which showed that direct stimulation of Barrington's nucleus induced bladder contractions while administration of CRF inhibited contractions [5], we also began to study corticotrophin-releasing factor (CRF) levels in Barrington's nucleus as a result of social stress.

Our prior studies have shown that the administration of the calcineurin inhibitor cyclosporine A (CsA) prevented stress-induced voiding dysfunction from developing; however the treatment did not change the levels of CRF mRNA in Barrington's nucleus [6]. In the clinical setting, we are faced with children who have already presented with established voiding dysfunction. We now present data that show the short- and long-term persistence of stress-induced abnormal voiding patterns following removal from the stressor. Given our prior work showing a role for the calcineurin—NFAT pathway in stress-induced voiding dysfunction, we hypothesized that treatment with CsA after stress exposure would improve or normalize the voiding phenotype.

Materials and methods

Protocols were reviewed and approved by the Institutional Animal Care and Use Committee at our hospital animal facility. All animals were housed with 12-h light/dark cycles at 21 °C. Food and water for all treatment groups was provided *ad libitum*.