PNPase inhibition as a novel and effective treatment for chronic bladder pain

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ABSTRACT

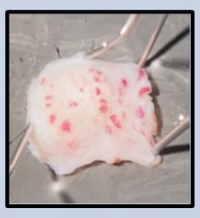
Chronic visceral pain disorders, such as bladder pain syndrome/interstitial cystitis (BPS/IC), are among the most difficult types of pain to treat and response to treatment is often negligible. Evidence suggests that oxidative stress and inflammation may play important roles in the pathophysiology of a number of these disorders. The enzyme purine nucleoside phosphorylase (PNPase) is important for the metabolism of 'tissue protective' purines to 'tissue-damaging' purines that generate free radicals (e.g., reactive oxygen species or ROS). Our preliminary studies reveal that inhibition of PNPase (oral treatment with 8-aminoguanine (8-AG) yields significant improvement in a bladder-centric model of BPS/IC (cyclophosphamide, CYP). The aim of this study uses the CYP model to validate a non-opioid based target, namely PNPase for the treatment of BPS/IC.

METHODS

Adult Sprague Dawley rats were divided into the following groups: 1) control; 2) CYP (75 mg/kg/i.p. every 3 days; sacrificed day 8) and CYP treated with 8-AG (5 mg/kg/day oral administration in drinking water beginning 14 days prior to start of CYP with daily dose monitored). The Institutional Animal Care and Use Committee has approved all procedures. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). We utilized metabolic cage to assess bladder function and behavioral assessment (von Frey microfilaments) to assess mechanical allodynia. After sacrifice, urinary bladders were collected, and tissues were assessed for gross visual inspection for inflammation and biomarkers for pain/inflammation (using western immunoblotting).

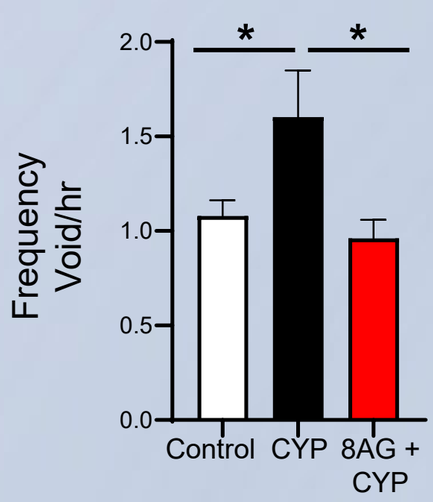
8-AG prevents gross inflammation of the urinary bladder (e.g., increased petechiae hemorrhage)

CYP-bladder



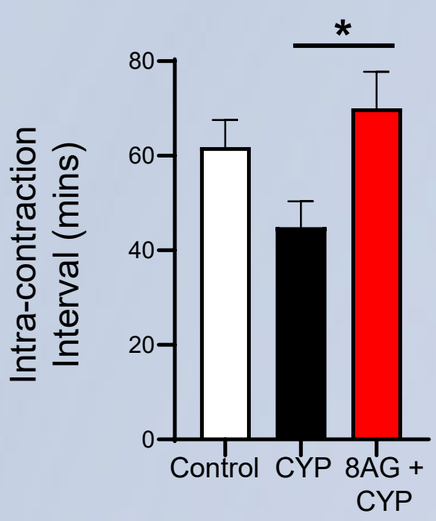
8AG-treated CYP bladder

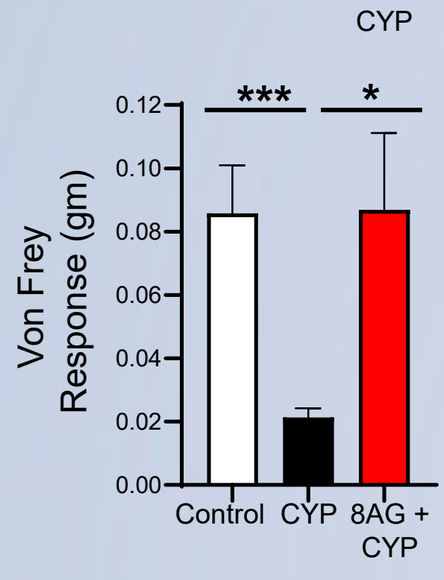
8-aminoguanine reverses voiding dysfunction and behavioral response to mechanical stimuli



Voiding frequency is significantly increased 149% after CYP treatment. The time between voids is decreased 27% in CYP treated rats.

CYP-induced changes in bladder function are restored to control levels by treatment with 8AG

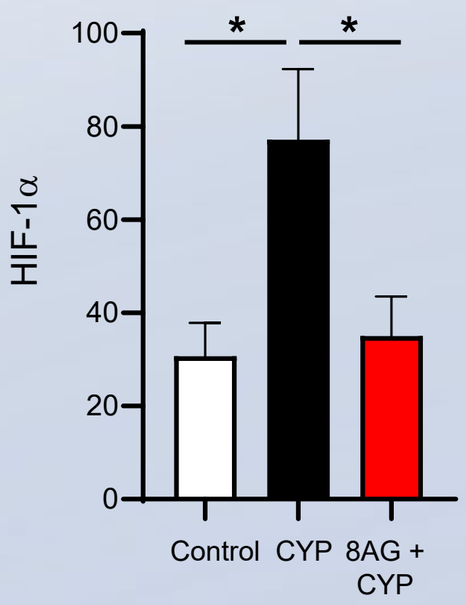




CYP treated rats exhibit significant changes in abdominal mechanical allodynia (*e.g., pain from stimuli which not normally provokes pain*).

Visceral allodynia was restored to control levels with 8-AG

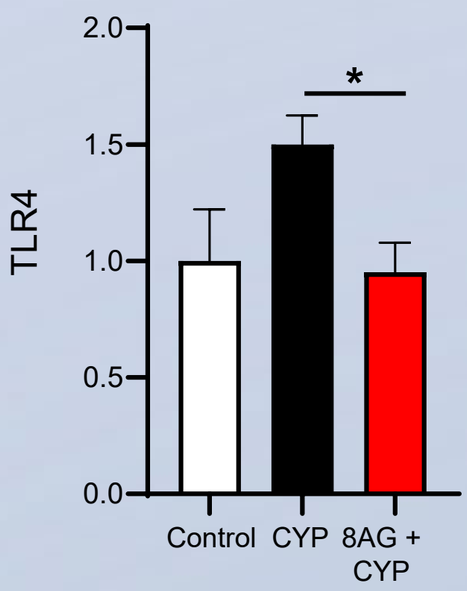
RESULTS



Hypoxia-inducible factor 1-alpha (HIF-1α) is a key signaling molecule which is increased due to tissue hypoxia and pro-inflammatory stimuli.

HIF-1α is significantly increased (252%) in the CYP-bladder.

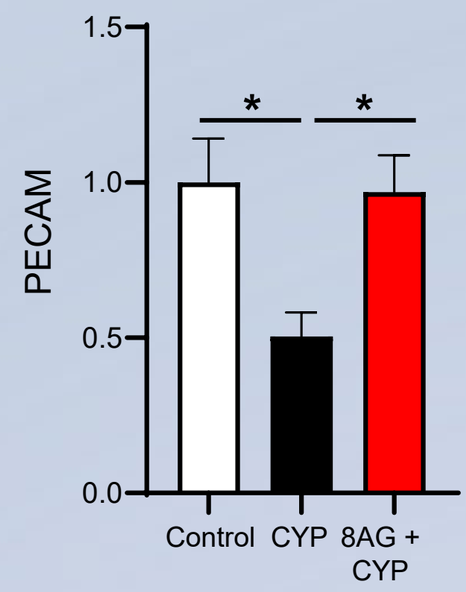
8-AG treatment prevents the increased expression of HIF-1α as compared to control



Toll-like receptor 4 (TLR-4)-mediated inflammatory responses are likely to play a role in chronic pain conditions and may play a central role in BPS/IC.

TLR-4 is increased (150%) in the CYP-bladder.

Upregulation of TLR-4 in CYP-bladder is normalized by 8-AG



Platelet-endothelial cell adhesion molecule-1 (termed PECAM or CD31) is a signaling molecule vital to regulating inflammatory responses.

PECAM is significantly reduced (50%) in the CYP-bladder. A reduction in PECAM may lead to prolonged inflammation.

8-AG treatment restores PECAM expression to control levels

CONCLUSIONS

Emerging evidence has revealed that alterations in the enzyme purine nucleoside phosphorylase (PNPase) reflects the participation of oxidative injury and cellular damage. PNPase products generated either within the target lower urinary tract (LUT) cell or remotely could damage LUT cells via ROS production. In sum, our preclinical findings support the use of PNPase inhibitors as a new class of drug therapy with corrective and restorative actions at the cellular level to improve bladder structure and function and reduce pain behavior and inflammation in a bladder-centric animal model for BPS/IC. Thus, while BPS/IC can stem from multiple causes making a single drug ineffective, we believe that targeting a single enzyme, PNPase, will restore the purine dysregulation thereby reducing inflammation and free radical formation while increasing antioxidant capacity.