

Kullmann F A¹, Wolf-Johnston A¹, Kanai A¹, Shiva S¹, Chelimsky T², Rodriguez L³, Birder L¹

1. University of Pittsburgh, PA, USA, 2. Medical College of Wisconsin, WI, USA, 3. University of Southern California, CA, USA

MIND-BODY INTERACTIONS: AUTONOMIC AND MITOCHONDRIAL DYSREGULATION PLAY KEY ROLES IN PBS/IC

Hypothesis / aims of study

There is substantive evidence supporting a role for chronic stress in the development, maintenance and even enhancement of functional bladder disorders such as painful bladder syndrome/interstitial cystitis (PBS/IC). More than half of patients with PBS/IC report daily or constant pain and urinary frequency, which are exacerbated by stressful circumstances. Stress can alter the sympathetic nervous system, whose activity is augmented in PBS/IC patients. While the etiology of functional disorders such as PBS/IC are not known, alterations in the bladder urothelium have been detected in both patients and animals diagnosed with PBS/IC. The urothelium not only forms a high-resistance barrier but also functions as an integral part of a 'sensory web' that can sense changes in their environment including those produced by chronic stress and via release of transmitters, can activate sensory nerves.

There is exciting new evidence that mitochondrial dysfunction and oxidative stress have been implicated in chronic pain as well as autonomic dysregulation. In fact, mitochondrial dysfunction may also be a final common pathway in the pathophysiology of many diseases and disorders that are impacted by chronic stress. Recent evidence shows that individuals with chronic conditions have impairment in mitochondrial pathways. *Our novel concept is that abnormal processing of chronic stress involving autonomic and mitochondrial dysregulation can negatively impact urothelial signalling, leading to altered sensations and pain in patients with PBS/IC.*

The aim of this study was to examine the influence of chronic stress and autonomic signalling on mitochondrial dysfunction in bladder urothelium.

Study design, materials and methods

This aim was investigated in an animal model consisting of a rat genetically predisposed to stress (Wistar Kyoto), exposed to psychological stress (chronic water avoidance or WAS).

- Female Wistar Kyoto rats (~200g; 3-4 month old) were exposed to WAS for 1h per day for 10 days and sacrificed on day 11 (versus handled groups as controls).
- To assess the impact of sympathetic signalling some rats were treated with guanethidine which depletes catecholamines (50 mg/kg, i.p.; every other day starting 2 days prior to WAS and throughout the WAS protocol) or the adrenergic ($\alpha 1/\alpha 2$) antagonist, phenoxybenzamine (2 mg/kg i.p. every day starting the day WAS protocol was initiated and throughout the WAS protocol).
- Bladders were collected from deeply anesthetized rats and utilized for cell culture and western blot per previously published methods.
- Cultured urothelial cells (UTC) from control and WAS rats were loaded with various intracellular dyes to examine functional (intracellular calcium or mitochondrial) responses. These included: fura-2AM (to measure intracellular calcium concentration, $[Ca^{2+}]_i$), Dihydrorhodamine 123 - DHR123 (to measure reactive oxygen species - ROS) and Tetramethylrhodamine methyl ester - TMRM (to measure mitochondria membrane potential, Ψ_m ; Figure 1).

Results

Our findings reveal that WAS UTC exhibit a more depolarized Ψ_m (~30%; decreased staining of the fluorescent indicator TMRM indicating a loss of viability; Figure 1) compared to control UTC. We also have evidence for higher baseline $[Ca^{2+}]_i$ and inability to buffer $[Ca^{2+}]_i$ after a stimulus (i.e., mitochondria uncoupler carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone or FCCP 5-10 μM). Both guanethidine and phenoxybenzamine treatment of WAS animals normalized these alterations, supporting sympathetic nervous system involvement.

There was no difference in basal ROS production in between WAS or control UTC. In contrast, ROS generation in response to stressors (H_2O_2 : 100 μM) was significantly higher in WAS UTC. ROS were also generated in both control and WAS UTC by stimulation of α -ARs with phenylephrine (10 μM) but not of β -ARs with isoproterenol (1 μM).

We also find that chronic stress correlates with increased mucosal protein carbonylation as well as an increase in pAMPK often termed the metabolic gate keeper of the cell. In addition, we find an increase in mitofusin 2, which is thought to suppress cell proliferation and exert apoptotic effects. In all cases, we see a normalization in these targets in WAS animals treated with guanethidine or phenoxybenzamine.

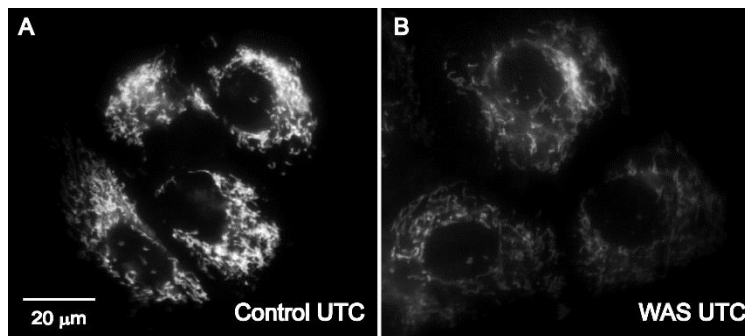


Figure 1. Examples of cultured UTCs from control (A) and WAS (B) rats, loaded with the mitochondria Ψ_m indicator TMRM. The intensity of the dye is proportional to the Ψ_m ; less bright cells indicate depolarized Ψ_m (suggesting a loss of cell viability).

Interpretation of results

While a number of factors are likely to contribute to chronic pain disorders, accumulating evidence suggests that altered cellular metabolism (i.e. mitochondrial functions) plays a key role. Mitochondrial uptake of calcium is important for cell survival and regulating release of mediators (such as ATP and ROS). The mitochondrial membrane potential Ψ_m is an important parameter to assess the functional state of these organelles. Disturbances in Ψ_m could result in alterations of cell metabolism, increased ROS production and altered calcium homeostasis; all of which were observed in WAS UTC.

Mitochondria are responsible for the majority of oxygen consumption and represent the major source of reactive oxygen species in all cells including urothelium. Chronic stress (with augmented levels of stress mediators such as norepinephrine) can lead to a prolonged change in mitochondrial properties. For example, the increase in ROS can result in protein carbonylation which is used as a biomarker for oxidative stress. In turn, WAS UTC may increase AMPK, which can activate anti-oxidant defenses.

Many of these changes are prevented by treatment with agents that either deplete catecholamines (guanethidine) or block alpha-adrenergic receptors (phenoxybenzamine) supporting a role for the sympathetic nervous system. Taken together, chronic stress results in persistent sympathetic-mediated effects that alter cellular (mitochondrial) memory, and this may result in changes to the urothelial barrier and signalling functions.

Concluding message

Chronic stress triggers a number of changes that ultimately can exacerbate or predispose to disease such as PBS/IC. These novel findings support the concept that abnormal processing of psychological stress involves a complex urothelial-cell signalling network which can be influenced by sympathetic and mitochondrial dysregulation. Mitochondria play an important role in controlling the life and death of a cell. Thus, our results have broad implications for functional pain syndromes and provide new information regarding how changes in mitochondrial functions in chronic stress may influence sensory signalling. Mitochondria targeted therapies may hold future promise to restore abnormal signalling in functional pain disorders such as PBS/IC and may contribute to improvement of symptoms in these patients.

Disclosures

Funding: RO1 DK57284, R37 DK54824 and Pilot Research Grant from the Interstitial Cystitis Association (ICA) to LAB **Clinical Trial:** No **Subjects:** ANIMAL **Species:** rat **Ethics Committee:** Institutional Animal Care and Use Committee at the University of Pittsburgh, which adheres to NIH Guidelines for the Care and Use of Laboratory Animals. The University of Pittsburgh is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC).