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André Ferreira Canelas

Sympathetic nervous system and visceral pain: the case of the bladder pain syndrome

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Sympathetic nervous system and visceral pain: the case of the bladder pain syndrome

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Aos meus pais,

À Bia,

À Raquel,

Os verdadeiros mestres da minha vida.

Sympathetic nervous system and visceral pain: the case of the bladder pain syndrome.

By

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Abstract.

Aim: To review and summarize available data on the possible influence of sympathetic nervous system in Bladder Pain syndrome/Interstitial Cystitis (BPS/IC) pathogenesis.

Methods: A search for relevant articles was performed in the Medline, EMBASE, Web of Science and Cochrane Library electronic databases. Only English language publications since 1900 to January 2014 were selected.

Results: The true causes of BPS/IC remain elusive. However, several causes have been recently proposed, including epithelial dysfunction, deregulation of the immune response and neurogenic inflammation. The contribution of the of sympathetic nervous system to BPS/IC has been traditionally disregarded but there is strong evidence for a sympathetic role. Indeed, published reports show that rat models of chronic adrenergic stimulation reproduced many of clinical aspects and typical histological findings of BPS/IC patients.

Conclusion: The reports found and analyzed in the present review indicate that several factors may contribute to the etiology of BPS/IC. Particularly, the sympathetic nervous system emerges as one of the main contributors to BPS/IC pathogenesis. Its influence may underlie at least some of the multiple problems reported by BPS/IC patients.

Key-words: Interstitial Cystitis, Bladder Pain Syndrome, Painful Bladder Syndrome, Sympathetic Nervous System, Adrenergic Fibers, Autonomic Nervous System, Neurotrophins; Mast cells; Urothelium.

Introduction

Bladder pain syndrome/interstitial cystitis (BPS/IC) is a poorly defined clinical condition. The prevalence in USA was suggested to reach 100/100 000 women (1). More recent European epidemiological surveys reported a prevalence of about 200-300/100 000 women (2). No cure exists yet for this disease and the etiology is unknown. The initial diagnostic criteria were proposed in 1987 by the National Institute of Diabetes and Kidney Disease (NIDDK) (3). These criteria are now obsolete as they miss more than 60% of cases. A more accurate definition of BPS/IC was recently proposed by the American Urological Association (AUA) for diagnosis and treatment. According to the AUA, BPS/IC is characterized by: "An unpleasant sensation (pain, pressure, discomfort) perceived to be related to the urinary bladder, associated with lower urinary tract symptom(s) of more than 6 weeks duration, in the absence of infection or other identifiable causes'' (4).

BPS/IC may be accompanied by other pathological conditions, not necessarily bladder – related. The most common comorbidities associated to BPS/IC include irritable bowel syndrome (IBS), fibromyalgia, chronic fatigue syndrome, Sjogren's syndrome, chronic pelvic pain, anxiety disorders, migraine, allergies, asthma, sleep disorders and other syndromes not related to the bladder (5). Epidemiologic studies suggest that only 7% of BPS/IC patients have none of those co-morbid syndromes (6). Conversely, patients with these non-bladder syndromes have an higher probability of developing BPS/IC in the future, and this probability is proportional to the number of associated syndromes (7). Interestingly, these non-bladder syndromes shares many of the characteristically findings of BPS/IC, such as, the worsening during stressful situations and the possible involvement of urothelial and mast cells as well as the neurotrophins in their pathogenesis. These common features may suggest there is a common factor in their pathophysiology.

The true causes of BPS/IC remain elusive, with a variety of possibilities being indicated by several clinical and experimental studies. Therefore, the aim of this study was to review and summarize available data on this matter.

Methods

A search for relevant articles was performed in the Medline, EMBASE, Web of Science and Cochrane Library electronic databases with different combinations of the following key-terms: “Interstitial Cystitis”, “Bladder Pain Syndrome”, “Painful Bladder Syndrome”, “Sympathetic Nervous System”, “Adrenergic Fibers”, “Autonomic Nervous System”, “Neurotrophins” and “Mast cells”. Only English language publications since 1900 to 2014 January were included. Additionally, references of retrieved articles were scanned for any potentially relevant article.

Results

The pathophysiologic mechanisms underlying BPS/IC remain unclear. However, several causing agents have been proposed in recent years. These include epithelial dysfunction, deregulation of the immune response and neurogenic inflammation (8). A number of animal models have been used for the study of BPS/IC, which include administration of an irritant or immune stimulant (e.g. hydrochloric acid, turpentine, protamine sulfate, mustard oil, lipopolysaccharide and cyclophosphamide). In addition, cats with with feline interstitial cystitis and an experimental autoimmune cystitis (EAC) murine model have also been used. These animal models exhibit a number of functional and histological alterations of the bladder, comparable to those observed in in human BPS/IC (9, 10), and have been widely used to identify the putative cause of this syndrome. Available results point towards damage

of the urothelium, mast cells activation in the submucosa, synthesis of neurotrophins and sympathetic activation as key factors of BPS/IC.

Damage of the urothelium: a key factor in BPS/IC.

The urothelium is the specific epithelium lining of the distal urinary track. The tight and adherent junctions of its uppermost cells, the umbrella cells layer, together with a thin coating mucinous layer of glycosaminoglycans and glycoproteins, form an effective barrier that is essential for normal bladder function (11). It is now well established that the urothelium is not just a passive barrier. The close anatomical relation between the urothelium and afferent nerves, coursing in the lamina propria, suggests an interaction between them (11). Following mechanical or chemical stimulation, urothelial cells release various substances, including the neurotrophin nerve growth factor (NGF), adenosine triphosphate (ATP), acetylcholine, nitric oxide and prostanoids that can stimulate submucosal sensory nerves and cause pain (12). Urothelial cells also respond to the same substances either in an autocrine fashion or when released by activated nearby nerve fibres (13), which may lead to loosening of intercellular junctions and loss of urothelial integrity. A leaky urothelium has been observed in biopsies from BPS/IC patients and in bladder samples from animal models of this disease (13). The increase in urothelium permeability allows leaking of water, urea, pronociceptive and inflammatory substances, present in the urine, into the bladder wall, further activating sensory fibres and immune cells and leading to inflammation (12) and intense bladder pain, as described by patients.

Mast cell: a neuroimmunoendocrine connection.

Mast cells are immune cells that play an important role in various pathological conditions. In bladder biopsies from BPS/IC patients the density of activated mast cells was upregulated. The reason for this may reside in the increased levels of stem cell factor (SCF) in nerve endings of the bladder wall, an chemotactic factor for mast cells that stimulates their proliferation and secretion (14). In the bladder, mast cells were located very close to the submucosa nerve fibres (15), suggesting an interaction between these immune cells and bladder innervation. When activated, mast cells may release histamine, bradykinin, prostaglandins, leukotrienes, growth factors, cytokines and proteases (16). Most sensory fibres coursing the bladder wall express receptors to these proinflammatory elements. Activation of these receptors leads to sensitization of bladder sensory fibres, resulting in pain and urinary dysfunction present in BPS/IC patients and experimental animal with bladder inflammation (17, 18). In turn, when stimulated, these fibres may release a variety of neuropeptides that act on mast cells, further stimulating the activation of these immune cells (19). Therefore, there is a close-knit interaction between mast cells and nerve fibres, which may easily become uncontrolled and potentiated via positive feedback in BPS/IC.

In addition, mast cells may also be, at least partially, accounted for vascular remodelling of the bladder wall, also known to occur in BPS/IC (20) and animal models of this pathology (21). Activated mast cells are known to release tryptase that leads to microvascular leakage and activation of protease-activated receptors (PARs), expressed by urothelial cells, nerve fibres and immune cells, resulting in local inflammation (22). Likewise, vascular endothelial growth factor (VEGF), also released by mast cells, may induce hypervascularity and glomerulations (20), which are typical endoscopic findings found in BPS/IC patients (20, 22).

Interestingly, VEGF may further sensitize bladder sensory fibres as they express receptors for this vascular growth factor (23).

Neurotrophins in BPS/IC

Neurotrophins (NTs) are trophic factors, produced in peripheral tissues, required for neuronal survival (24). The urinary bladder is a major source of NTs, particularly of the two most abundant NTs, Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF) (24). The concentration of NGF is elevated in the bladder and urine of BPS/IC patients, positively correlating with the severity of symptoms (25). Urinary BDNF is also elevated (25). These high levels of NTs in the bladder are thought to result from increased release from the urothelium (26) and from immune cells present in the submucosa (27). The presence of higher NTs levels contributes to the sensitization of bladder sensory fibres. It is well known that the high affinity receptors of NGF and BDNF, tropomyosine-related kinase A (TrkA) and tropomyosine-related kinase B (TrkB) respectively, are present in bladder afferents (24) and their expression and activation state are increased in rats with chronic cystitis (28). Upon binding to their specific receptors, NTs may regulate the sensitization of bladder afferents either by direct interaction with membrane receptors or by regulating gene expression. For example, in sensory afferents NGF is known to quickly increase the expression of the ionic channel transient receptor potential vanilloid receptor 1 (TRPV1) (29) as well as lowering its activation threshold (30). In addition, NGF also upregulates the expression of the neuropeptide Substance P and BDNF. Upon stimulation, these afferents may release Substance P in the bladder, activating mast cells (19) and nerve fibres (31). BDNF is released at the spinal cord level, where it modulates excitatory neurotransmission

by phosphorylating specific subunits of the glutamate receptor, N-methyl-D-aspartate (NMDA), via intracellular activation of mitogen-activated protein kinases (MAPKs) (24) .

Given their importance in BPS/IC, modulation of NTs has been proposed as a measure to reduce pain and improve bladder function. NGF modulation with recombinant proteins or humanized monoclonal antibodies results in reduced pain and urinary frequency (32, 33). BDNF modulation has only been tested in animals also leading to a significant reduction in pain levels and urinary frequency (34, 35). However, the side effects observed in patients after NGF sequestration and in animals after intrathecal BDNF scavenging may preclude the clinical application of this strategy (33, 34)

The involvement of the sympathetic nervous system in BPS/IC

Stress and sympathetic activation have been shown to impair the immune, endocrine and nervous systems and can be an important factor in functional gastrointestinal (GI) and genitourinary (GU) disorders such as IBS and BPS/IC. Rats exposed to various types of stress (water avoidance, intruder stress) exhibit symptoms of bladder dysfunction including increased micturition frequency as well as anxiety-like behaviour (36, 37). Further, an exaggerated acoustic startle response has been demonstrated in both cats diagnosed with feline IC as well as in BPS/IC patients (38, 39).

The importance of the sympathetic nervous system in BPS/IC has been traditionally disregarded. However, the density of sympathetic nerve fibres is higher in bladder biopsies from BPS/IC patients (40) and the urinary concentration of catecholamines is also very high (41). Both parameters positively correlate with the severity of symptoms (42).

To better understand the putative role of the sympathetic nervous system in BPS/IC, our group was recently involved in a study analysing both human data and experimental results

(43). In BPS/IC patients noradrenaline levels in blood at resting conditions and in 24-hr urine were higher than in healthy controls. The TILT test revealed sympathetic system hyperactivity in the same patients (Table I).

These results are in line with other studies that demonstrated higher mean blood pressure and elevated heart rate during bladder hydrodistention in BPS/IC patients (44). Altogether, such results suggest that sympathetic hyperactivity may be an important, if not the key, cause of BPS/IC. To investigate a possible causal relation adrenergic overactivity and the bladder changes occurring in BPS/IC, we developed and animal model in which rats were submitted to daily subcutaneous administration of phenylephrine (PHE), a potent non-catecholamine, predominantly alpha adrenoreceptor agonist.

In rats submitted to chronic adrenergic stimulation behavioural signs of pain were obvious (Fig. 1) and accompanied by increased spinal Fos expression (a surrogate marker of pain at the spinal cord level), as well bladder and faecal hyperactivity.

Histological analysis of the bladder identified suburothelial mastocytosis and large areas of reduced urothelium thickness (Fig. 2A, 2B), similarly to what is typically observed in bladder biopsies from BPS/IC patients. Also, the expression of pro-apoptotic markers and a reduction of the expression of cytokeratin observed in that study (Figs. 2C-E), strongly suggests that sympathetic hyperactivation is the underlying mechanism responsible for urothelial dysfunction in BPS/IC (45). Our clinical and experimental observations indicate that sympathetic hyperactivity is, at least, partially responsible for BPS/IC. The proposal of this novel hypothesis may open new opportunities for a better understanding of this syndrome and the design of more efficient therapeutic strategies.

Discussion

BPS/IC is a heterogeneous syndrome and available studies pinpoint a myriad of factors that may contribute to its etiology. The possible contribution of the sympathetic nervous system was initially raised following observations in BPS/IC patients of elevated blood pressure and heart rate during bladder distention (44) and increased sympathetic innervation (46). Our results confirmed sympathetic dysfunction in BPS/IC patients, assessed both by the TILT test and the high urinary noradrenaline concentration. Chronic administration of phenylephrine closely replicated a number of typical findings in BPS/IC patients, including strong visceral pain, urothelial dysfunction, mast cell infiltration, and visceral dysfunction. It is, thus, very likely that sympathetic hyperactivity is at the root of BPS/IC.

Indeed, it is possible that an initial hyperactivation of the sympathetic system may initiate a cascade of events that leads to BPS/IC. Noradrenaline release in the bladder wall may active mast cells and lymphocytes, known to express alpha-adrenoreptors (47). These cells may release pro-inflammatory elements and neurotrophins that may act on bladder sensory nerves, causing pain and bladder dysfunction and also lead to severe urothelial damage, that also contributes to pain and bladder hyperactivity (15, 18, 40). This sympathetic mediated release of mast cell mediators could also explain the worsening of most of BPS/IC clinical symptoms in stressful conditions.

An important observation is that chronic administration of PHE also lead to faecal hyperactivity, which is reminiscent of colonic hyperactivity, or even the presence of IBS (43), in BPS/IC patients. Although the occurrence of cross-organ sensitization can be demonstrated following experimental bladder or colon inflammation and reflect dichotomy sensory innervation of both organs (48), one could speculate that both conditions may result

from sympathetic hyperactivity. In fact, patients with irritable colon also present increased sympathetic innervation and urinary noradrenaline (49).

Conclusion

BPS/IC is considered a multifactorial disease. The role of the sympathetic nervous system has been poorly investigated but its hyperactivity may be a crucial event for the pathophysiology of BPS/IC. This may have important clinical implications as different experimental and clinical approaches may be used with BPS/IC patients. Further research is necessary not only to identify the triggering event leading to sympathetic activation but also to design more efficient therapeutic strategies.

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Tables and Figures

Table I. Sympathetic overactivity in IC/BPS patients.

	Control	BPS/IC
ΔSDPP	57.2 ±723.0	24.2 ± 18.2*
rMSSD	6.3 ±2.8	5.6 ± 8.4
BRS	7.1 ±3.8	7.7 ± 8.2
24 hr urinary NA (mg/day)	47.8 ±17.8	102.1 ± 43.7*
Plasmatic NA (supine) (µg/ml)	204.3 ±46.6	397.0 ± 36.4***
Plasmatic NA (upright) (µg/ml)	578.3 ±69.0	596.3 ± 30.4

Standard deviation of the P wave intervals (ΔSDPP); Root mean square successive differences (rMSSD); Baroreflex sensitivy (BRS); Noradrenaline (NA).
Mean values of observations obtained during TILT test, and noradrenaline quantification in 24 hr urine samples and plasma.
ΔSDPP was used to record the increment in sympathetic activity. rMSSD and BRS were used to evaluate the parasympathetic system.
*P<0.05.
***P<0.001
Adapted from Charrua et al. (2013)

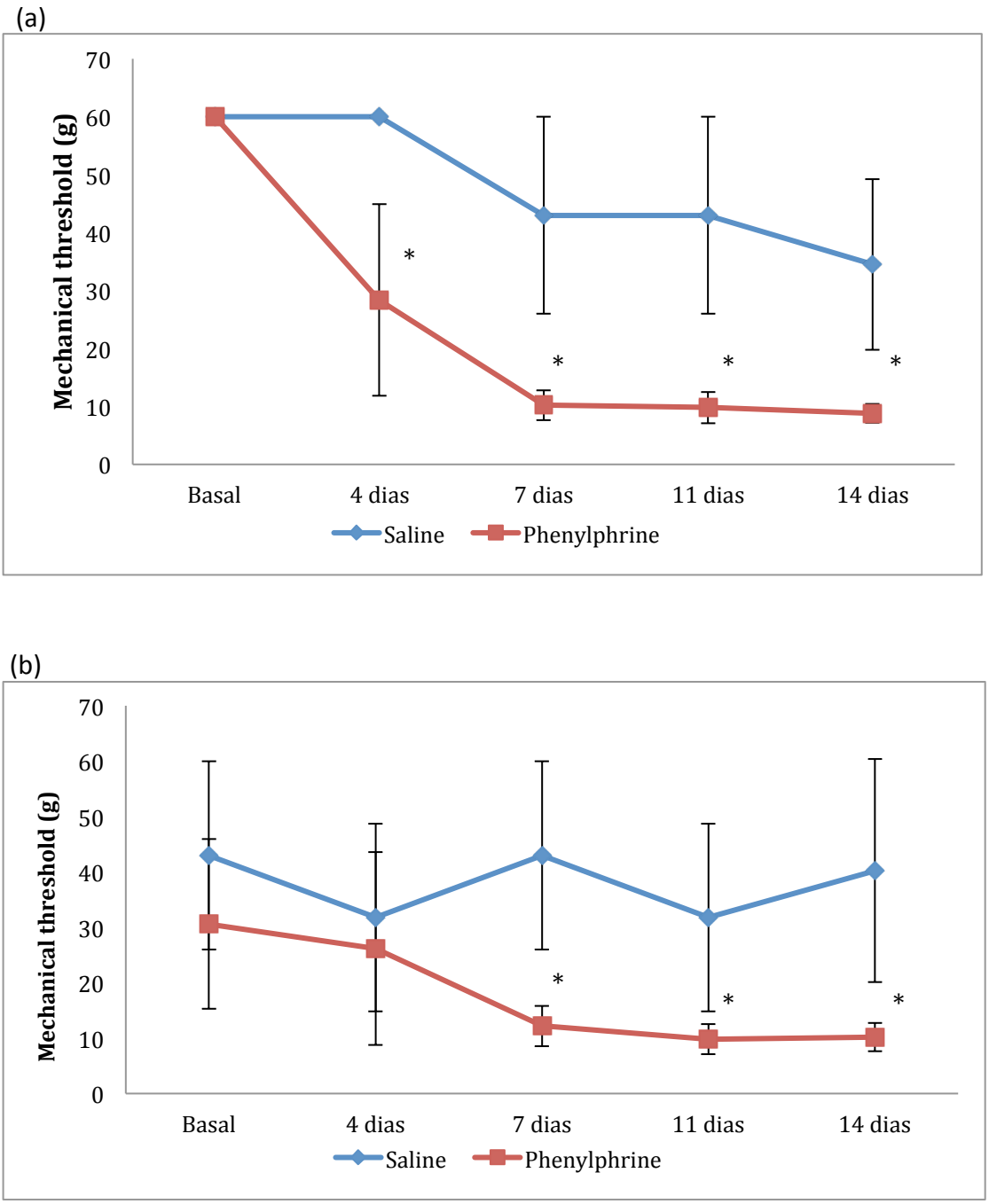

Fig. 1. Mechanical sensitivity of the lower abdomen (a) and hindpaw (b) after chronic phenylephrine treatment. The group treated with phenylephrine (PHE) had a significantly lower threshold both in the abdomen (a) and.in hindpaw (b). The increase in the mechanical sensitivity was evident from the 1st week of treatment onwards ($p<0.05$ versus non-treated rats). This indicates the presence of visceral pain.
*$P<0.01$ vs saline treatment; repeated measures ANOVA followed by Student-Neumman-Keuls.
Adapted from Charrua et al. (2013)

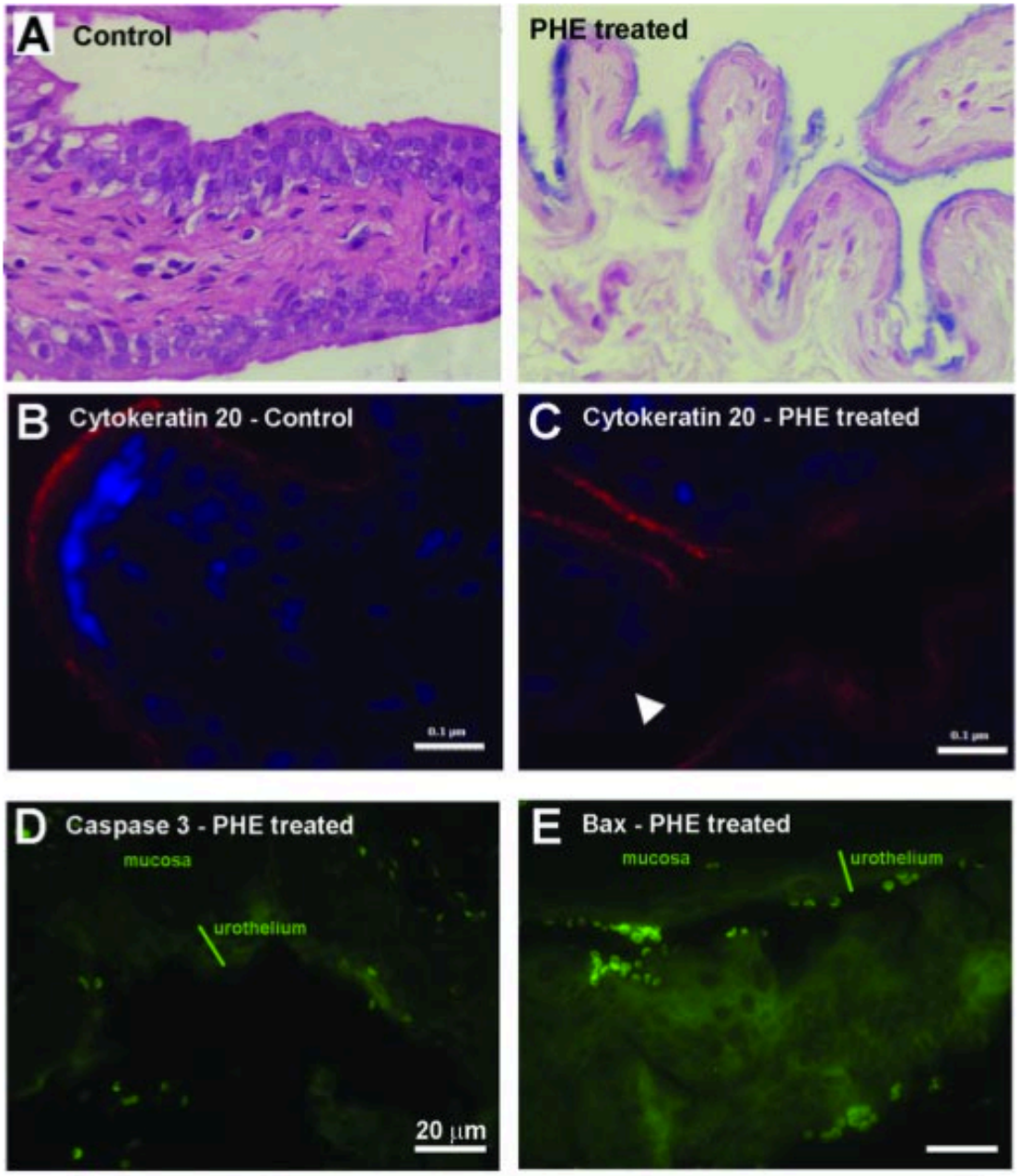

Fig. 2. Urothelial changes following chronic administration of phenylephrine. A: Haemotoxilin/eosing staining showed that compared with controls, PHE treated rat bladder shows a reduced thickness of urothelium. **B/C:C**ytokeratin 20-positive umbrella cells (in red) that are decreased or absent in areas of PHE treated group bladders (nuclei are stained in blue). **D/E:** Staining of Caspase 3 and BAX pro-apoptotic proteins was strongly increased in PHE-treated rats. Adapted from Charrua et al. (2013)

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Este trabalho é de todos nós. Muito obrigado a todos.

Anexos

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