Microcirculatory aspects of neurogenic bladder syndrome

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Ph.D. Thesis

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Szeged

2020

LIST OF FULL PAPERS RELATED TO THE SUBJECT OF THE THESIS

- I. **Járomi P**, Szabó A, Garab D, Bodnár D, Uhercsák G, Boros M, Hartmann P. Experimental studies on microcirculatory inflammatory reactions of the urinary bladder. *Magy Seb.* 2012;65(4):184-190. doi: 10.1556/MaSeb.65.2012.4.3. (in Hungarian)
- II. **Járomi P**, Garab D, Hartmann P, Bodnár D, Nyíri S, Sántha P, Boros M, Jancsó G, Szabó A. Capsaicin-induced rapid neutrophil leukocyte activation in the rat urinary bladder microcirculatory bed. *Neurol Urodyn.* 2018;37(2):690-698. doi: 10.1002/nau.23376. (IF 2018: 2.36)
- III. **Járomi P**, Banyó T, Boros M, Papp F, Szabó A. Clinical aspects and therapeutic possibilities of neurogenic bladder. *Orv Hetil.* (*in press*) (IF: 0.53)

ABSTRACTS RELATED TO THE SUBJECT OF THE THESIS

- IV. **Járomi P**, Hartmann P, Szabó A, Garab D, Lakatos Á, Boros M. Comparison of antigen-dependent and independent inflammatory reactions in the rat urinary bladder. XVth Congress of the Hungarian Society of Urology, Debrecen, 2010, Experimental Urology Session, P 12 (in Hungarian)
- V. **Járomi P**, Hartmann P, Garab D, Bodnár D, Boros M, Szabó A. Inflammatory microcirculatory changes in different experimental models of cystitis in rats. 9th World Congress on Urological Research, 15-17 September 2011, Innsbruck, Austria, WCUR11-0076

LIST OF ABBREVIATIONS

CGRP	calcitonin gene-related peptide
CYP	cyclophosphamide
DSD	detrusor sphincter dyssynergia
HC	hemorrhagic cystitis
IC	interstitial cystitis
ICAM-1	intercellular adhesion molecule-1
IR	ischemia/reperfusion
IVM	intravital videomicroscopy
NO	nitric oxide
NK1	neurokinin-1
PMN	polymorphonuclear leukocyte
TRPV1	transient receptor potential vanilloid type 1

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SUMMARY

The microcirculatory aspects of inflammatory disorders are of importance in the pathology of the urinary system. Our first aim was to perform a comparative analysis of the microcirculatory responses of the urinary bladder in infectious and non-infectious inflammatory animal models with direct or indirect endothelial damage. To this end, we compared the local microcirculatory consequences of experimental interstitial (IC) cystitis and hemorrhagic cystitis (HC) with those of bladder ischemia/reperfusion (IR). We found that not only IR, where direct endothelial damage is present, but also HC and IC, where microcirculatory inflammatory reactions are secondary after urothelial and interstitial damage, are associated with manifest polymorphonuclear leukocyte (PMN)–endothelial cell interactions. This finding confirms the common role of PMN-mediated microcirculatory reactions in the pathogenesis of bladder diseases.

The overexpression of transient receptor potential vanilloid type 1 (TRPV1) has been demonstrated in IC cases as well as in cases of neurogenic bladder. In addition, TRPV1 agonists have also previously been used during pharmacological management of these diseases. Therefore, our next aim was to examine the microcirculatory effects of local capsaicin treatment (the archetypical TRPV1 agonist). In our study, capsaicin induced rapid increases in PMN leukocyte rolling and adhesion and in adhesion molecule expression in the postcapillary venules of the urinary bladder, which was prevented by neonatal sensory chemodenervation with capsaicin and competitive TRPV1 antagonism. The effect of specific receptor antagonist therapy showed that TRPV1-induced calcitonin gene-related peptide (CGRP) release initiates the PMN–endothelial cell interaction by promoting leukocyte rolling, but adhesion is influenced by both CGRP and substance P.

Our final goal is to implement medical research in clinical practice. Depending on the underlying cause, neurogenic bladder can manifest in both overactive and underactive forms. Diagnosis and treatment of both manifestations of neurogenic bladder remain a challenge in urology practice because disease severity cannot easily be assessed and the most common symptoms are non-specific. For this reason, our last aim was to provide an algorithm that aims to facilitate rapid and efficient diagnosis and assessment of disease severity. This may also aid in decision making on the potential pharmacological and invasive therapeutic approaches to protecting the upper urinary tract by maintaining low pressure values in the bladder.

1. INTRODUCTION

Inflammatory microcirculatory reactions

All the essential elements of inflammation (*rubor, tumor, dolor, and calor*) can be traced back to phenomena occurring at the level of the microcirculation. The microcirculation delivers cells and molecules that transmit inflammatory signals to affected tissues, and, as a result, the function of the endothelial cells within the microvessels is typically altered. In this case, however, activation of the endothelium during the “classic inflammatory response” to antigens is not an initiating factor, but rather a resultant one. In other cases, such as in hypoxia/reoxygenation processes involving oxidative damage, the endothelial layer appears to be the main target of damage and injury of the endothelium is therefore a primary event. The outcome will be similar in both cases, resulting in endothelial activation and/or endothelial cell dysfunction. This is characterized by an altered release of endothelium-derived vasoactive mediators and an expression of adhesion molecules involved in the cellular phase of inflammation (Leppilahti M 2002; Starkman JS 2008) with a resultant increase in cell-to-cell interactions (including leukocyte–endothelial cell interactions) which further aggravate the microcirculatory processes (Yadav SS 1998). In the pathogenesis of endothelial dysfunction, the major role of free radical-mediated reactions can be assumed; however, the release of proteolytic enzymes (e.g. elastase, cathepsin G, and protease III) activated during reperfusion are likewise of considerable importance (Hisadome Y 2007). During the process of microcirculatory inflammation, polymorphonuclear leukocytes (PMNs) are also activated, showing an increased expression of adhesion molecules which (in concert with endothelial activation) results in tissue migration of PMNs bringing about additional local tissue damage (Leick M 2014). Microvascular dysfunction caused by ischemia/reperfusion (IR) injury will eventually (1) manifest in characteristic microcirculatory reactions marked by decreases in (a) functional capillary density, (b) red blood cell velocity, (c) venular and arteriolar diameters, and (d) macromolecular leakage; however, it will also (2) bring about microcirculatory inflammatory reactions that are characterized by PMN-related reactions in the postcapillary venules (Bajory Z 2002a).

1.1. Types of sterile urinary bladder inflammation and their research models

One of the most common forms of bladder inflammation of non-infectious origin is interstitial cystitis (IC), also known as irritable bladder syndrome (Driscoll A 2001), where the tissue damage is initiated in the epithelial layer, causing ulcerous histological changes and a characteristic deep rupture through the mucosa. Since distinctive inflammatory reactions are present at the affected site, it was proposed (by the European Society for the Study of Interstitial Cystitis) that the term “Hunner’s ulcer” previously used for this morphological alteration be replaced with the term “Hunner’s lesion,” thus indicating the characteristic functional (inflammatory) aspect of the underlying pathophysiological events (van de Merwe JP 2008). The interstitial component of the barrier lesion is most probably responsible for the clinical signs and symptoms (Yoshimura N 2014), but hypoperfusion may also play a role in the background of ulcer development in IC (Lavelle J 2002). It has been proposed that a urothelial dysfunction (a local mechanism) can raise the number of substances released by the urothelium (e.g. adenosine triphosphate and nitric oxide (NO)) and neurogenic inflammation, which is associated with mast cell activation (Smith CP 2005; Veselá R 2012). These local changes have been shown to cause afferent hyperexcitability (Yoshimura N 2014) with increased numbers of C-fiber afferents and of mRNA-level receptors of substance P (neurokinin-1 receptor, NK1) in the mucosa of the bladder in patients with IC (Sukiennik A 2004). Therefore, sensitization of bladder afferent pathways and altered sensory processing in the spinal cord have both been proposed as potential mechanisms that induce IC (Yoshimura N 2013).

Another common inflammatory disease of the bladder is hemorrhagic cystitis (HC), which is a relatively common iatrogenic side-effect of alkylating chemotherapeutic agents (deVries CR 1990). Acrolein, the toxic metabolite of cyclophosphamide (CYP), elicits an inflammatory response of the urothelium, which subsequently also affects the interstitium and microvessels (Cox PJ 1979). In animal models of HC, afferent neurons in the lumbosacral dorsal root ganglion also showed an increased expression of neuropeptides, such as substance P (Vizzard MA 2001), marking a potential neurogenic component of this condition. Apart from tissue infiltration of PMNs, the importance of other inflammatory factors (sensory neuron and mast cell activation) has been demonstrated in both forms of bladder inflammation (IC and HC) (Lyon D 1982; Nazif O 2007; Sakthivel SK 2008), but the role of microcirculatory reactions in the pathogenesis of IC is unclear.

The urinary bladder (like other organs) also undergoes hypoxia/reoxygenation injury, although at a lower rate of incidence than those of the diseases above, with the related microcirculatory reactions having been characterized in standardized experimental settings (Bajory Z 2002a). Potential causes of bladder IR include embolization, thrombophlebitis, vasospasm, arteriosclerosis, pregnancy, pelvic tumors, vascular ligation during operations, and trauma (Love A 1978). The morphological and functional consequences may manifest in decreased bladder compliance, reduced capacity and contractility, blood-urinary barrier impairment, edema, smooth muscle degeneration, and necrosis/apoptosis (Yamaguchi O 2014). Upon intermittent ischemia of the urinary bladder, the vessel endothelium is a primary and direct target of IR injury. In cases of IC and HC, however, the injury is initiated at the epithelial/urothelial surface and further propagated towards the interstitium, causing a potential secondary (indirect) involvement of the microvasculature.

1.2. Neurogenic bladder syndrome (recently termed “Neurogenic Lower Urinary Tract Dysfunction”)

Urine storage and urination disorders

In the case of normal urinary function, urine is excreted by the kidneys, stored in the bladder, and then emptied at appropriate intervals. In the storage phase, the bladder is in a relaxed state, but as the filling volume increases, urinary excretion occurs voluntarily in response to the sensation of the bladder filling in the central nervous system. This is brought about by the constriction of the bladder muscles in the presence of simultaneous relaxation of the outer sphincter. Disorders or impairment of these functions, however, manifest in various urinary storage and emptying disorders, which are currently defined as Lower Urinary Tract Symptoms. Urinary storage disorders are classified into three main forms, i.e. stress incontinence, hyperactive bladder, and neurogenic bladder (Burkhard FC 2020) (Figure 1).

Figure 1. Classification of urinary storage disorders (based on http://www.hbcs.hu/uploads/jogszabaly/2312/fajlok/EEM_szakmai_iranyelve.pdf; 2016. EüK. #6. EMMI guideline)

Etiology of neurogenic bladder

Neurogenic bladder is a collective term used for all urinary storage and emptying disorders with neurological diseases in the etiology (see below) with the most common symptoms being stress incontinence and overactive bladder. Not only is the bladder involved in these processes, but also the sphincter and posterior urethra (collectively referred to as the “bladder outlet”). Therefore, this complex disorder is currently also termed “Neurogenic Lower Urinary Tract Dysfunction”). Neurogenic bladder covers a wide range of neurological dysfunctions, affecting millions of patients worldwide. The underlying cause can be diverse, ranging from local functional disorders (e.g. autonomic dysreflexia and detrusor sphincter dyssynergia (DSD)) or local (e.g. after pelvic surgery, peripheral) nerve injuries, but can also originate from upper and lower motoneuron injuries (spinal lesions) or degenerative neurological diseases. The most common causes of neurogenic bladder include spina bifida (neurogenic bladder symptoms occurring in more than 95% of cases) (Torre M 2011), spinal cord injuries (in ~90% of these cases) (Cameron AP 2010), multiple sclerosis (in 50–80% of these cases) (Nicholas RS 2009; Nortvedt MW 2007), stroke, Parkinson’s disease, diabetes, infections, and poisoning

(<https://www.urologyhealth.org/urologic-conditions/neurogenic-bladder>). These diseases result in disturbances in coordinated function of the bladder and sphincter due to alterations/injury in the afferent and efferent nerves, the spinal cord, or central nervous system.

The two major clinical manifestations of neurogenic bladder. The importance of timely and accurate differential diagnosis

It is important to note that “Neurogenic Lower Urinary Tract Dysfunction” manifests in characteristically different symptoms based on the localization of the underlying neurogenic cause. The most common symptom is urge incontinence, which may be associated with urinary incontinence and/or urinary retention, depending on the localization of the disease. Based on the etiology or the localization of the lesion (Figure 2), there are two major manifestations of the disease: the overactive (we call it Type #1 here) and the underactive (termed Type #2) forms of bladder activity, both of which are accompanied by reduced bladder compliance. Upper motoneuron (suprapontine and infrapontine/suprasacral) lesions cause detrusor overactivity (Type #1 neurogenic bladder), while lower motoneuron (sacral/infrasacral and cauda equina) injury and injury to the peripheral nerves result in flaccid bladder with impaired function of detrusor muscle and decreased sensation of fullness (Type #2 neurogenic bladder). Lesions between the pontine and the sacral urinary center cause DSD. The formation of a reflex in the sensory branch (automation via capsaicin-sensitive C-afferent fibers) may also play a role in the pathogenesis of neurogenic bladder (Blok B 2020).

The consequences of both of these nervous system processes will ultimately lead to similar consequences manifesting in increased bladder pressures, vesico-ureteral reflux, and resultant ureteral and renal dilatations, hydronephrosis, and pyelonephritis (which also predispose to infections and stone formation) and finally renal failure (Amarenco G 2017; Panicker JN 2015).

Figure 2. Functional classification of lower urinary tract disorders associated with typical neurogenic lesions (Groen J 2016)

As noted above, both forms of neurogenic bladder may lead to irreversible damage of the upper urinary tract and significantly affects life quality, causing deteriorations in mental health and work performance, and sleeping disorders. Accurate and timely diagnosis is also highly important to prevent permanent kidney damage, recurrent infections, or even urosepsis (Allio BA 2016). Differential diagnosis is particularly difficult because overactive bladder can originate not only from neurogenic causes, but can also be idiopathic or myogenic in origin, with symptoms that are not specific to neurogenic bladder. Although the underlying neurological causes are usually detectable, a patient-specific, individualized therapy is called for usually based on the nature and severity of the symptoms. Therefore, accurate diagnosis of the severity of urodynamic changes is extremely important to prevent renal damage.

1.3. The role of transient receptor potential vanilloid type 1 (TRPV1) receptors in physiological and neurogenic inflammatory processes

C-fiber chemosensitive primary sensory neurons innervate a variety of organs and tissues and express the TRPV1 receptor (Szallasi A 1999). The role of TRPV1 receptors is not yet completely understood, but physiological modulatory functions and protective roles have been attributed to them. TRPV1 receptors modulate neurogenesis (Jin K 2004), thermomodulation (Gavva NR 2007), or organ function, i.e. the urinary bladder (Birder LA 2001, 2002; Franken J 2014). The activation of the TRPV1 receptor by noxious stimuli results not only in the generation of nociceptive impulses transmitted to the central nervous system, but also in the release of neuropeptides from the stimulated sensory nerve endings. Neuropeptides released from sensory nerves (e.g. calcitonin gene-related peptide, CGRP, and substance P) initiate an array of local tissue reactions, including vasodilatation and plasma extravasation, i.e. a neurogenic inflammatory response. There is ample evidence of the role of chemosensitive primary afferents in the transmission of nociceptive impulses and mediation of neurogenic inflammation in a variety of organs and species, including humans (Moskowitz MA 1993; Nathan JD 2002; Szallasi A 1999; West MA 1996). Neurogenic inflammation refers to the flare (Bayliss WM 1901) and plasma extravasation (Jancsó N 1967) which is brought about by electrical stimulation of unmyelinated afferent nerves, for instance (Kenins P 1982). TRPV1 receptors are generally activated by inflammatory mediators, including lipoxygenase products, bradykinin, and prostaglandins (Davis JB 2000; Julius D 2001; Zygmunt PM 1999). The role of TRPV1 activation has been confirmed in pathophysiological processes of neurogenic inflammation in animal models of colitis, cystitis, arthritis, and bronchitis (Baluk P 1999; Kihara N 2003), but these receptors have also been shown to play a protective role against IR injury in the heart (Wang L 2005) and kidney (Chen L 2014) and in allergic dermatitis (Bánvölgyi A 2005).

In the urinary bladder, TRPV1 is expressed richly in the afferent fibers, the majority of which contain peptides (Szallasi A 1999), and these are in close contact with urothelial cells. Furthermore, urothelial cells also express these receptors (Birder LA 2001), and these cells are therefore most probably implicated in sensory signaling (Li M 2011), including sensory perception of elongation/expansion of the bladder (Everaerts W 2010). This particular class of sensory nerves significantly contributes to the regulation of urinary bladder functions, including the micturition reflexes in both animals in general and humans

(de Groat WC 2009; Fowler CJ 1992; Juszczak K 2010). Moreover, several pathological conditions of the urinary bladder, including neurogenic detrusor overactivity, idiopathic detrusor overactivity, and bladder outlet obstruction involve changes in C-fiber afferent mechanisms in animals (Kitagawa Y 2013; Yokoyama O 2010) and in humans (Cruz F 1997; de Groat WC 2009; Fowler CJ 1992). TRPV1 is also critically involved in inflammatory processes of the urinary bladder. In patients suffering from IC/“bladder pain syndrome”, the severity of the clinical symptoms and inflammation correlated with the enhanced expression of TRPV1-immunoreactive nerve fibers in the urinary bladder (Liu L 2007) and urothelial cells from the overactive human bladder exhibit a high capsaicin sensitivity (Burnstock G 2006). The involvement of TRPV channels has been demonstrated in cases of overactive bladder and neurogenic detrusor overactivity (Fowler CJ 1992; Kuo HC 2006).

TRPV1 is involved in the transmission of urinary bladder pain and the application of TRPV1 agonists, and by eliminating capsaicin-sensitive sensory nerves, it is of therapeutic use in the treatment of pathological pain conditions of the urinary bladder. TRPV1 agonists (capsaicin and resiniferatoxin) have been used for many years to alleviate chronically painful conditions, such as diabetic neuropathy and cancer pain (Knotkova H 2008; Lazzeri M 1998, 2004; Oliveira R 2019). These effects may be related to reduced abnormal urinary reflex functions (Maggi CA 1986, 1989) and partly due to desensitization and destruction of TRPV1 receptors (Bley K 2012; Dasgupta P 1998). These treatments were, however, often performed on an empirical basis (Avelino A 2006).

1.4. TRPV1-related microcirculatory reactions

TRPV1 activation is known to bring about characteristic microcirculatory reactions in several organs. The activation of the TRPV1 receptor through the release of vasoactive peptides, such as CGRP and substance P, from the sensory nerves triggers an extremely rapid elevation in blood flow and an increase in permeability in different organs, e.g. the skin (Boros K 2016; Cao T 2000), the dura (Dux M 2003), and the pancreas (Grady EF 2000). Neurotransmitters released from perivascular nerves are generally implicated in the concept of neurogenic inflammation (Seifert H 1985; Sternini C 1992; Zimmerman BJ 1992).

TRPV1-mediated vasodilatory effects are partially linked to the release of CGRP in the dura mater and the skin (Dux M 2003; Williamson DJ 1997). Another, indirect mechanism involves the enhanced activation of endothelial Ca^{2+} -dependent NO synthase and resultant NO release elicited through a TRPV1-dependent rise in intracellular Ca^{2+} . This is supported by findings that show that the release of NO could be inhibited by pretreatment with the TRPV1 receptor antagonist capsazepine *in vitro* (Ching LC 2011) and by sensory denervation *in vivo* (Poblete IM 2005). Edema formation elicited by the activation of TRPV1 receptors, however, is linked to the release of substance P and its binding to endothelial NK1 receptors, which lead to an increase in vascular permeability (Cao T 2000) and PMN infiltration (Hood VC 2000) in the skin (Boros K 2016) and the pancreas (Bhatia M 1998), where the dose-dependent increase in plasma extravasation evoked by substance P and capsaicin could be inhibited by capsazepine (Boros K 2016; Grady EF 2000) or by NK1 receptor antagonism (Nathan JD 2002).

Even though TRPV1 agonists are used in the therapeutic management of urinary bladder disorders, the potential acute microcirculatory side-effects of TRPV1 activation have not yet been examined. The present study was therefore designed to examine the effects of capsaicin, the archetypal TRPV1 agonist, on PMN–endothelial cell interactions in the microcirculatory system of the rat urinary bladder, using intravital videomicroscopy (IVM) and immunohistochemistry. The contribution of sensory nerves and the sensory neuropeptides substance P and CGRP to these inflammatory phenomena was studied with the aid of selective sensory chemodenervation and pharmacological antagonists.

2. MAIN GOALS OF THE STUDIES

- The microcirculatory aspects of non-infectious forms of cystitis have not yet been characterized. Therefore, we aimed to perform a comparative analysis of inflammatory microcirculatory responses of the bladder in animal models under conditions with presumed direct or indirect endothelial damage. More importantly, we compared the microcirculatory consequences of IC and HC with those of bladder IR.
- Even though TRPV1 agonists were used in the therapeutic management of urinary bladder disorders, the potential acute microcirculatory side-effects of TRPV1 activation have not yet been examined. We thus also aimed to investigate the effects of capsaicin, the archetypical TRPV1 agonist, on PMN–endothelial cell interactions in the microcirculatory system of the rat urinary bladder, using IVM and immunohistochemistry. The contribution of sensory nerves and the sensory neuropeptides substance P and CGRP to these inflammatory phenomena was analyzed with the aid of selective sensory chemodenervation and pharmacological antagonists.
- Depending on the cause, neurogenic bladder manifests in both overactive and underactive forms. The accompanying symptoms (i.e. urge incontinence +/- urinary incontinence +/- urinary retention) are not specific to neurogenic bladder, and the clinical cases also show diverse severity. Further, pathophysiological changes in the lower urinary tract could retrogradely damage the ureter and the kidneys. For these reasons, our further aim was to design and propose an algorithm for a rapid and efficient diagnosis and severity assessment of neurogenic bladder in the practice of urology in Hungary. This also aids in choosing a pharmacological or invasive therapeutic approach to protect the upper urinary tract by maintaining low pressure values in the bladder.

3. MATERIALS AND METHODS

The experimental part of the present thesis is based on two series of animal studies. In the first experimental series (Study 1), the microcirculatory consequences of common urological diseases were studied in the rat urinary bladder. In the second experimental study (Study 2), the microcirculatory side-effects and the underlying receptor-dependent mechanisms of topical capsaicin were examined. In the review article (Study 3), we aimed to set up a diagnostic algorithm to facilitate a proper diagnosis of disease severity and analysis of the urodynamic consequences of neurogenic bladder.

3.1. Animals (Studies 1–2)

The experimental studies were approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), under license numbers I-74-1/2009. (Study 1) and V./144/2013 (Study 2). These studies were conducted in compliance with EU Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and the National Institutes of Health guidelines for the use of experimental animals.

3.2. Surgical procedures (Studies 1–2)

Healthy adult male Sprague–Dawley rats (with body weight ranging from 280–330g) were anesthetized intraperitoneally with an initial dose of sodium pentobarbital (45mg kg^{-1}) and animals were placed in a supine position on a heating pad to maintain body temperature at $36\text{--}37^{\circ}\text{C}$. The trachea was cannulated to facilitate spontaneous respiration. Catheters were also inserted into the carotid artery and jugular vein for continuous measurement of blood pressure (Statham P23Db transducer) and for administration of continuous infusion ($10\text{ml kg}^{-1}\text{ h}^{-1}$ Ringers lactate) and of supplementary doses of pentobarbital (if necessary), and the injection of fluorescent dyes (see later). There were no significant differences in the blood pressure among the different experimental groups (see later) at any time point during the microcirculation measurements (data not shown).

The urinary bladder was prepared for IVM examination using a modified method developed by Bajory et al. (Bajory Z 2002a). Following a midline laparotomy, the bladder was exposed and the median umbilical ligament resected. A polyethylene catheter (ID

0.28mm, OD 0.61mm; Smiths Medical, UK) was later inserted into the bladder dome and fixed by a ligature (via a 3-0 braided polyglycolic acid thread). Since the catheter insertion caused nearly complete emptying of the bladder, 0.5ml of a body-warm 0.9% NaCl solution was injected to provide a similar, standard baseline filling state of the bladders in all of the experiments. In pilot studies, this intravesical pressure ($<7\text{mmHg}$) caused no microcirculatory disturbances in the bladder. The remaining abdomen was covered with Saran wrap to avoid fluid and heat loss and drying out of the surface of the abdominal organs.

3.3. Study design and experimental protocols (Studies 1–2)

Study 1

In Study 1, the experiments were conducted in four groups ($n=21$). In the first group, bladder ischemia (IR; $n=5$) was performed with reversible ligation of the aa. vesicales for 60 min, and then microcirculatory consequences were examined after an additional 30 min (Bajory Z 2002a). In the second group, the early phase of IC was induced by 30-minute intravesical instillation of protamine sulfate ($2\text{mg}/200\mu\text{l}$ in physiological saline; $n=6$), which causes selective urothelial damage, under ketamine & xylazine anesthesia (90mg kg^{-1} and 10mg kg^{-1} , respectively) by sterile puncture of the bladder dome after laparotomy (Chopra B 2005). Following protamine sulfate exposure, the bladder was washed with sterile saline and the abdomen was sealed in two layers under sterile conditions. Inflammatory reactions were examined 24 hours after induction. In the third group, HC was modeled, where animals were treated with a high dose of CYP intraperitoneally (40mg kg^{-1} ; $0.1\text{ml}/100\text{g}$, $n=5$) (Vera PL 2008). Microcirculation studies were performed after 24 hours. Sham-operated animals underwent laparotomy with their bladders instilled with physiological saline (sham-operated group, $n=5$).

Study 2

The aim of the experiments in Study 2 was to examine the microcirculatory inflammatory consequences of a 15-min topical application of capsaicin on the bladder surface. We used two major sets (see Figure 3). In Set 1 ($n=34$), the effects of TRPV1 receptor antagonism and the effects of ablation of TRPV1-positive nerves (achieved with the neonatal administration of capsaicin) were examined. Briefly, newborn male Sprague–Dawley rats were injected subcutaneously with a single dose of 50mg kg^{-1} capsaicin or its vehicle (6%

ethanol and 8% Tween 80 in saline) on Day 2 of life under ether anesthesia. Neonatal capsaicin treatment has been shown to produce a selective degeneration of chemosensitive afferent nerves which are sensitive to capsaicin and express the TRPV1 receptor (Jancsó G 1977). The eye-wiping test, using a 1% solution of zingerone dripped into the eyes of rats on the day of the experiment, was used to demonstrate the elimination of TRPV1-expressing afferent nerves (Jancsó N 1967).

In Set 2 (n=35), the effects of selective CGRP and NK1 receptor antagonists were studied by monitoring the capsaicin-induced cellular inflammatory changes. All topical treatments were performed by placing pieces of Gelaspon® sponge (Chauvin Ankerpharm GmbH, Berlin, Germany) soaked in their respective compound(s) on the anterior surface of the bladder for 15 min.

Figure 3. The time sequence of treatments and measurements in experimental groups in Sets 1 and 2 in Study 2. The topical treatments with capsaicin (CAP, 50µM) or other compounds (see below) were performed at the anterior surfaces of the bladders for 15 min. In Set 1, the TRPV1 receptor antagonist capsazepine (CZP, 200µM topically) and ablation of the TRPV1-positive nerves (achieved with the neonatal administration of CAP) were applied. In Set 2, the selective CGRP receptor antagonist CGRP₈₋₃₇ (10µM) and the NK1 receptor antagonists RP67580 (10µM) were topically applied together with CAP to investigate the consequences of selective CGRP and NK1 receptor antagonism. Here, IVM

= intravital microscopic examinations of leukocyte–endothelial interactions in postcapillary venules of the bladder.

In the first set of experiments in Study 2, the animals were placed in one or the other of the following experimental groups: the anterior surface of the bladder was topically treated with capsaicin or its vehicle by dripping 100µl of the solution on a Gelaspon sponge, which was placed on the bladder dome for 15 min in the first two groups (vehicle and capsaicin groups, n=6 and n=10, respectively). We examined the effect of the specific TRPV1 inhibitor capsazepine on the microcirculatory changes in two groups, where capsazepine was topically applied just before the administration of capsaicin or its vehicle (vehicle+capsazepine and capsaicin+capsazepine groups, n=6 each). These findings were compared with those obtained in rats (n=6) treated with capsaicin as neonates (Bajory Z 2002a). In the second set of experiments, the effects of the capsaicin receptor antagonist capsazepine on the capsaicin-induced inflammatory changes were studied. During these experiments (the second set), the microcirculatory effect of the CGRP and NK1 antagonism was examined and CGRP₈₋₃₇ (10µM) and RP67580 (10µM) were applied on the bladder surface in the presence (capsaicin + CGRP₈₋₃₇, n=5; capsaicin + RP67580, n=7) and in the absence of topical capsaicin (vehicle + CGRP₈₋₃₇, n=6; vehicle + RP 67580, n=8) (50µM). After a 15-min exposure, the sponge was removed from the bladder surface and thoroughly rinsed off with warm physiological saline and covered with a Saran wrap for the remainder of the duration. IVM recordings were made 15, 30, and 45 min after topical treatments. This overall 60-min observation period was chosen because accumulation of the fluorescence tracers in the urine with resulting deterioration in the contrast of the images did not permit a longer observation period.

3.4. Intravital microscopy (Studies 1–2)

The anterior surface of the bladder microcirculation was visualized by IVM (Zeiss Axiotech Vario 100HD microscope; Carl Zeiss GmbH, Jena, Germany; 100-W HBO mercury lamp; Acroplan 20x water immersion objective). The animals received intravenous injections of fluorescein isothiocyanate (FITC)-labeled erythrocytes (Ruh J 1998) (0.2ml; Sigma-Aldrich Co, St. Louis, MO, USA) for the verification of the intact perfusion of the microvascular bed under examination (using the 490/525nm filter set of the microscope). PMNs were stained by injecting rhodamine 6G intravenously (0.2%, 0.1ml; Sigma-Aldrich Co, St. Louis, MO, USA) and examined with a 526/555nm filter set

(Figure 4). Images were recorded with a charge-coupled device video camera (Teli CS8320Bi, Toshiba Teli Corporation, Osaka, Japan) attached to an S-VHS video recorder (Panasonic AG-MD 830; Matsushita Electric Industrial Co., Tokyo, Japan) and a personal computer.

Figure 4. Representative micrographs showing the sequence of PMN–endothelial interactions in three consecutive images (Panels A–C) recorded using intravital microscopy (recording rate: 20 frames/s). A segment of the postcapillary vein in the urinary bladder under examination is surrounded by lines in Panel A. The movement of rhodamine 6G-labeled PMNs (marked a–e) is demonstrated frame by frame relative to a dashed line. Stationary (adhesive) PMNs are marked by an ellipse. The bar in Panel C denotes a 100 μ m scale, and this applies to all the photomicrographs.

3.5. Analysis of microcirculation data (Studies 1–2)

A quantitative evaluation of the microcirculatory parameters was performed off-line via a frame-to-frame analysis of the videotaped images taken for IVM (IVM Software; Pictron Ltd., Budapest, Hungary). Red blood cell velocity (μm^{-1}) was measured in five different fields of view and five capillaries at each measurement time point in the experiment (average of 25 measurements were calculated). PMN–endothelial cell interactions were analyzed in four or more postcapillary venules within a minimum of three observation fields per rat (choosing the most representative vessels). All of the evaluations were performed by two investigators. Rolling PMNs were defined as cells moving with a velocity of less than 40% of that of the erythrocytes in the centerline of the microvessel and observed passing through the vessel segment under observation within 30s, expressed as the number of cells per mm per sec (Massberg S 1998). Adherent PMNs were defined as cells that did not move or detach from the endothelial lining within an observation period of 30s and are expressed as the number of cells per mm^2 of endothelial surface, calculated using the diameter and length of the vessel segment. In this study, postcapillary venules

with a diameter of 15–20 micrometers were used, and the average vessel length was about 80 micrometers.

3.6. Immunohistochemistry (*E-selectin and ICAM-1*) (Studies 1–2)

E-selectin and intercellular adhesion molecule-1 (ICAM-1) are endothelium-derived adhesion molecules that play a potential role during the course of leukocyte rolling and adhesion onto the endothelium within the postcapillary venules; hence, we decided to measure their expression in the bladder in response to capsaicin. Forty-five minutes after topical application of capsaicin on the urinary bladder, the organ was fixed by immersion in a fixative containing 4% paraformaldehyde in a phosphate buffer (0.1M, pH=7.4) for two hours at 4°C. Tissue samples were then transferred into a phosphate buffer until further processing. Frozen sections 15µm in thickness were cut with a cryostat and prepared for the demonstration of E-selectin and ICAM-1 immunoreactivity using the indirect double-labeling immunohistochemical technique. The sections were incubated overnight with mouse anti-rat anti-ICAM-1 (clone 1A29; BD Pharmingen, San Jose, CA, USA; 1:1000) and goat anti-rat E-selectin (R&D Systems, Minneapolis, MN, USA; 1:1200) primary antibodies dissolved in phosphate-buffered saline containing 0.3% Triton X-100. These sections were then incubated with DyLight® 488-conjugated donkey anti-mouse and Cy3-conjugated donkey anti-goat secondary antibodies (both from Jackson ImmunoResearch Europe, Newmarket, UK). They were covered with a ProlongGold mounting medium containing DAPI. Immunostained sections were then examined under a Zeiss LSM 700 confocal microscope. One micrometer thin optical sections were collected via Z-stack capturing and the maximum intensity projection function was used to reconstruct an image of the 12µm thick block of the tissue. Photomicrographs of control and capsaicin-exposed specimens were taken under identical conditions following a systemic random sampling method. Three bladders per group, three representative sections per bladder, and 5–9 regions per cryosection were selected for a quantitative evaluation. The observer obtained and evaluated photomicrographs of bladder specimens from the control and treated groups in a blinded manner. The line profile measurement tool in the ImagePro Plus 6 image analysis software package (Media Cybernetics, USA) was used to collect relative pixel intensity data from the postcapillary venule profiles (n=24–30 for each experimental group).

3.7. Methods used to develop a diagnostic algorithm for neurogenic bladder (Study 3)

We created an algorithm for the diagnosis of neurogenic bladder syndrome, which includes diagnostic interventions in the order of their proposed sequence, including specific information provided by the actual step (concerning classification and disease severity), based on the current literature data and our own clinical experience.

3.8. Statistical analyses

The minimum number of animals per group was determined based on the “PS: Power and Sample Size Calculator” software package. Data analysis was performed with the SigmaStat statistical software package (Jandel Corporation, San Rafael, CA, USA). In Study 1, differences between groups were assessed with the one-way ANOVA test, followed by the Bonferoni *post hoc* test. In Study 2, changes in microcirculation variables within and between groups were analyzed with the two-way repeated measures ANOVA test, followed by the Holm–Sidak test. Immunohistochemistry data were analyzed using the two-way ANOVA test. All data values are expressed as means $\pm$ standard error of the mean (SEM), and *P* values < 0.05 were considered statistically significant.

4. RESULTS

4.1. Changes in PMN rolling and adhesion in the postcapillary venules of the urinary bladder in different urological models of bladder inflammation (Study 1)

In the first experimental study, direct damage to the bladder endothelium caused by IR resulted in about seven-fold higher values in the number of rolling PMNs as compared to those in the sham-operated group in postcapillary venules (Figure 5A). Twenty-four hours after protamine sulfate instillation, this difference was approximately three-fold; it was approximately five-fold 24 hours after CYP treatment (Figure 5A). We observed a significant increase in PMN adhesion in all of the challenged groups, which reached a similar extent (an approximately seven-fold difference when compared to the sham group) in all the groups (Figure 5B).

Figure 5. Leukocyte rolling (A) and adhesion (B) in postcapillary venules of the urinary bladder in sham-operated animals and in animals treated with protamine sulfate (PS) or cyclophosphamide (CYP) or subjected to 60 min of ischemia followed by 30 min of reperfusion (IR). Values are presented as means $\pm$ SEM. Here, * $P < 0.05$ vs. sham.

4.2. Changes in red blood cell velocity in the capillaries of the urinary bladder in different urological models of bladder inflammation (Study 1)

Among the different challenges, only IR resulted in significant differences in red blood cell velocity as compared to those after a sham operation (Figure 6). Specifically, IR evoked approximately 30% lower values than those seen in sham animals, whereas red blood cell

velocity values were similar to those of the sham group in the IC and HC models (induced by protamine sulfate (PS) and CYP treatments, respectively).

Figure 6. Red blood cell velocity in bladder capillaries in sham-operated animals and in animals treated with protamine sulfate (PS) or cyclophosphamide (CYP) or subjected to 60 min of ischemia followed by 30 min of reperfusion (IR). Values are presented as means $\pm$ SEM. Here, $*P < 0.05$ vs. sham.

4.3. The effect of selective sensory chemodenervation and TRPV1 antagonism on the capsaicin-induced microcirculatory changes of the urinary bladder (Study 2)

Topical application of capsaicin triggered a marked increase in the degree of PMN–endothelial interactions in the postcapillary venules of the urinary bladder. Both primary (rolling, Figure 7A) and secondary (adhesion, Figure 7B) interactions were significantly enhanced within 15 min after topical treatment of capsaicin and remained elevated during the entire observation period of 45 min. Life-long sensory chemodenervation by neonatal capsaicin treatment completely prevented the capsaicin-induced vascular changes and indicated that the vascular effects of capsaicin are not direct, but are mediated through the release of vasoactive agents from the sensory nerves. Likewise, the co-administration of capsazepine, a competitive TRPV1 antagonist, markedly inhibited the intravascular inflammatory changes elicited by capsaicin. Lastly, neither capsazepine nor the vehicle for capsaicin produced any changes in PMN-mediated reactions.

Figure 7. Time course of changes in leukocyte rolling (A) and adhesion (B) in the postcapillary venules of the bladder after a 15-min topical exposure to capsaicin or the vehicle. In other groups, TRPV1 receptor antagonism (via capsazepine, 200 μ M) and the ablation of TRPV1-positive nerves by neonatal capsaicin treatment were also applied. Values are presented as means $\pm$ SEM. Here, * P < 0.05 vs. baseline; # P < 0.05 vs. vehicle.

4.4. The effects of CGRP and NK1 receptor antagonists on TRPV1 activation-induced microcirculatory reactions of the urinary bladder

In the urinary bladder, the capsaicin-induced increase in leukocyte rolling was prevented with the topical co-administration of the specific CGRP receptor antagonist CGRP₈₋₃₄ (see Figure 8A), but not by the NK1 receptor antagonist RP67580. However, PMN adhesion was significantly reduced by both the CGRP and the NK1 receptor antagonists (Figure 8B).

Figure 8. Time course of changes in leukocyte rolling (A) and adhesion (B) in postcapillary

venules of the bladder following a 15-min topical exposure to capsaicin or the vehicle. The effects of the selective CGRP antagonist CGRP₈₋₃₇ (10 μ M) and the NK1 receptor antagonists RP67580 (10 μ M) are also shown. Values are presented as means $\pm$ SEM. Here, * P < 0.05 vs. baseline; # P < 0.05 vs. vehicle.

4.5. The effect of capsaicin on the expression of E-selectin and ICAM-1 in the urinary bladder microvasculature

In control urinary bladders, we noticed a faint-to-moderate staining of small venules with antibodies raised against E-selectin (Figure 9A and C) and ICAM-1 (Figure 9B and C). Topical application of capsaicin on the urinary bladder resulted in a massive increase in both E-selectin (Figure 9D and F) and ICAM-1 (Figure 9E and F) immunoreactivities in small venules. A quantitative evaluation of the staining intensity revealed a significant rise in the relative pixel intensity for both E-selectin (control: 8.11 ± 2.97 ; capsaicin: 35.14 ± 3.45 ; $P < 0.05$) and ICAM-1 (control: 5.45 ± 2.71 ; capsaicin: 33.59 ± 3.24 ; $P < 0.05$) immunostaining.

Figure 9. Confocal photomicrographs illustrating the immunohistochemical localization of ICAM-1 (A and D) and E-selectin (B and E) in urinary bladders 45 min after exposure to the vehicle (A–C) and capsaicin (D–F). Note the marked capsaicin-induced increase in immunostaining of both ICAM-1 and E-selectin localized to venules in the images at the bottom. The merged photomicrographs (C and F) illustrate the close colocalization of ICAM-1 and E-selectin immunoreactivities. The bar in F denotes a 50 μ m scale, and this applies to all photomicrographs.

4.6. Diagnostic possibilities of neurogenic bladder

As a first step, an accurate medical history should provide information about the already recognized neurological problem(s) and about the incidence and characteristics of urinating disorders (Type #1 reflex bladder with dribbling; Type #2 weak urinary stream) (Table 1). Questionnaires (e.g. King's Health Questionnaire, EQ-5D, Sf-qualiveen, Patient Perception of Bladder Condition, Patient Perception of Intensity of Urgency Scale, and Work Productivity and Activity Impairment) and urine diaries aid in an objective assessment of the severity of symptoms (Pannek J 2009). A routine urological physical examination usually shows urinary retention, can reveal costo-lumbar sensitivity in both cases, and, therefore, does not show specific results. Routine urine examinations (tracing pyuria and bacteriuria) and bladder and kidney ultrasound examinations (showing retention and damage of the upper urinary tract) may detect signs of an advanced stage of the disease, but are not capable of distinguishing between the two types of neurogenic bladder. Urodynamic examinations (cystometry and uroflowmetry) (Figures 10 and 11) (Amarenco G 2017; Cameron AP 2016; Marks BK 2014; Nosseir M 2007; Schäfer W 2002), however, are indispensable to differentiating between the two major forms of neurogenic bladder, but neurological (Podnar S 2001) and electromyographic examinations (Allert M 1973; Bacsu CD 2012) should also be conducted. Using cystometry, the functional state of the urinary tract is assessed invasively by analyzing pressure-volume curves in the urinary bladder (Amarenco G 2017; Schäfer W 2002). Using this method, functions of the detrusor muscle and sphincter are examined (during gradual filling and phases of strong and urgent urination stimuli), involuntary contractions can be detected, and bladder compliance is calculated (Type #1: involuntary contractions; Type #2 acontractile bladder can be detected). Based on the shape of the uroflowmetric recordings, DSD can be distinguished from urinary outflow obstruction using nomograms (Burkhard FC 2020). Video cystography is appropriate for detecting bladder neck dissynergism, internal sphincter, and bladder neck stenosis (Marks BK 2014).

Table 1. Recommended diagnostic algorithm for neurogenic bladder syndrome in the sequence of the proposed examination methods

	Type #1 patient: with overactive bladder (upper motoneuron lesion; injured above the lumbo-sacral region, e.g. stroke and trauma)	Type #2 patient: with flaccid bladder (lower motoneuron lesion; injured below the lumbo-sacral urinary center, e.g. trauma)
Pathomechanism of symptoms	Involuntary detrusor contraction, pressure rise, DSD	Underactive detrusor, urinary retention, reflux
Differential diagnosis	<ul style="list-style-type: none"> - (Idiopathic) hyperactive bladder - IC - Cystitis (and infections of the urinary system) - CYP-induced cystitis - Bladder tumor 	<ul style="list-style-type: none"> - Prostatic hyperplasia - Urethral stricture - Constipation - Pelvic tumor
1. Examination: history, clinical symptoms, questionnaires and urine diaries	Autonomic bladder (“reflex bladder”) +/- intermittent urination (in DSD)	+/- urge to urinate +/- frequent urination +/- involuntary loss of urine
2. Routine urological physical examination	+/- urinary retention +/- costo-lumbar sensitivity	+/- urinary retention +/- costo-lumbar sensitivity
3. Urine analysis	+/- pyuria +/- bacteriuria	Pyuria (+/-microscopic hematuria +/- bacteriuria)
4. Ultrasound (urinary bladder and kidney)	+/- urinary retention +/- pyelectasia	Urinary retention +/- pyelectasia
5. Cystometry (Figure 10)	Involuntary detrusor contractions during bladder filling, dissynergistic sphincter function	Pressure rise occurs at higher volumes during bladder filling
6. Electromyography	Decreased/no intentionally induced electrical activity, reflex activity unchanged	Decrease or complete absence of voluntary and reflex activity, denervation potential, fibrillation, fasciculation
7. (Video) Urodynamic investigation (uroflowmetry) (Figure 11)	Urination: lower volume is emptied, dribbling/intermittent urination may occur	Urination: decreased flow rate, prolonged urination, urination with abdominal pressure or Credé maneuver
8. Neurological examination	Intact bulbocavernosus reflex, increased rectal sphincter tone	Lesion of bulbocavernosus reflex, decreased rectal sphincter tone
9. Additional tests (for differential diagnosis (e.g. to exclude tumors)	Cystoscopy, CT (cysto-urethrography)	Renal scintigraphy, micturition cysto-urethrography, retrograde cystography (to detect reflux)

Figure 10. Typical cystometric images of upper (A) and lower (B–C) motoneuron lesions. A. Involuntary detrusor contractions with simultaneous rises in intravesical pressure (in a patient with DSD). B. No increase in intravesical pressure due to filling and no contractions (therefore, no spontaneous uroflow image can be provided). C. Urination under external pressure on the abdomen (source: own images).

Figure 11. Uroflowmetry chart of a healthy individual

5. DISCUSSION

5.1. Microcirculatory effects of urological diseases related to direct or indirect endothelial injury

IR injury causes functional injury in the urinary bladder, and microcirculatory changes play a potential role in this process. The causal relationship between functional and microcirculatory changes was confirmed in a study where ischemic preconditioning not only ameliorated the postischemic microcirculatory changes in the bladder, but also prevented deterioration in contractility (Hisadome Y 2007). In other models involving indirect endothelial damage (such as IC or HC), however, this relationship has not yet been clarified.

The aim of our experiments was to elucidate the reactions at the level of the microcirculation in models where endothelial activation/damage is not a primary phenomenon, but a secondary one. The exact etiology of IC is not fully understood, but several theories have emerged, including the role of infection, accumulation of toxic metabolites in the bladder, urothelial dysfunction, and neurogenic effects (Driscoll A 2001; Marcu I 2018; Parsons CL 2011; Sant GR 2001). Increased release of pro-inflammatory cytokines and chemokines as well as a beneficial effect of inhibition of their receptors have also been described (Malley SE 2002; Vera PL 2010), suggesting an important role for inflammatory responses in this scenario. In general, inflammatory cytokines modulate both immunological responses and sensory processes that may directly affect the regulation of urination as the basic function of the bladder (Rutkowski MD 2002; Vallejo R 2010). This line of reasoning is also supported by clinical observations, where increased inflammatory infiltration in IC patients has been reported (Sant GR 2007). Similarly, an increased chemotactic chemokine receptor signaling can be detected in a HC model, where inhibition of this pathway resulted in reduced bladder hyperexcitability, which is also associated with improved bladder compliance in rats (Arms L 2010).

When examining microcirculatory inflammatory reactions in models of indirect endothelial injury here, the extent of these changes were compared to those caused by IR injury (as a basis of comparison). It was shown previously in this condition that the disruption of microperfusion and the increase in microvascular permeability are associated with

increased cell-to-cell inflammatory responses (Bajory Z 2002a). Cell surface receptors responsible for PMN infiltration during reperfusion are well-known (Chamoun F 2000; Chopra B 2005) and PMN-induced damage could be influenced favorably by blocking antibodies used to inhibit PMN adhesion (Sasaki M 2007). It is also known that, in addition to urinary urothelium and muscle tissue, vasoconstrictive endothelin receptors located on endothelial cells also play a pronounced role in IR-induced microcirculatory changes in the bladder (Bajory Z 2002b). In the case of the bladder, reperfusion is characterized by hypoperfusion, and the classical *rubor* sign of inflammation (which corresponds to hyperemia at the level of the microcirculation) is not present in this organ. The explanation for the decreased flow rate could be derived from (1) the predominance of endothelium-mediated release of mediators toward vasoconstriction and/or (2) direct endothelial damage as well as (3) an increased edema formation during reperfusion (i.e. increased microvascular injury; Bajory Z 2002a).

The early phase of IC can be well modeled with intravesical instillation of protamine sulfate, which causes acute, selective damage to the urothelium (Lavelle J 2002) and reversibly inactivates the glycosaminoglycan layer (Tzan CJ 1993). The protamine sulfate-induced inflammatory responses are mediated through epithelial damage and a resultant increase in permeability (Lavelle J 2002). In patients with IC, severity of bladder inflammation correlated with not only pain and urgency scores, but also the density of TRPV1-immunoreactive nerve fibers (particularly with those in the suburothelial layer) (Liu BL 2014). Leukocyte invasion and mast cell activation have previously been reported in protamine sulfate-induced cystitis (Danisman A 2007; Stein PC 1996), but the related microcirculatory disorder was first described by our group here.

Our present experiments demonstrate that diseases not directly associated with endothelial damage cause microcirculatory disturbances similar to that of IR. This conclusion can be drawn based on our observations of cell-to-cell interactions in models of both IC and HC. The importance of microcirculatory changes in the pathogenesis of IC is also supported by observations where improvement in the bladder barrier function alleviated clinical symptoms with simultaneous reduction in the number of ulcers, suggesting an amelioration in the underlying circulatory disturbances (Lavelle J 2002). There are also assumptions regarding a release of irritating agents which increase epithelial permeability, thus inducing neurogenic inflammatory activation (Elbadawi AE 1996). Others hypothesize a relative

deficiency of NO production in the pathogenesis of the disease because a nitric oxide donor reduced the symptoms markedly (Moldwin RM 2002). Local free radical release can also play a role because the free radical scavenging melatonin (Sener G 2002) and dimethyl sulfoxide (Perez-Marrero R 1988) also had a beneficial effect on disease outcome. Close localization of mast cells and sensory neurons has been demonstrated in both irritable bowel syndrome and IC (Alexacos N 1999; Bauer O 2000; Theoharides TC 1996). In human studies with IC, significant changes in PMN-derived gene expression (Gamper M 2009) and increased tissue accumulation of PMNs (Cox PJ 1979; Erickson DR1997) as well as of the expression of the adhesion molecule ICAM-1 have been demonstrated (Leppilahti M 2002). Further, changes in leukocyte cytokine ligands were found in experimental IC (Sakthivel SK 2008).

CYP-induced HC is a very common non-infectious cystitis in clinical practice. This form of cystitis is the most widely used animal model of cystitis; it involves a significant transformation of urinary reflexes, causing symptoms similar to those of IC (Vizzard MA 2001). CYP-induced cystitis is characterized by marked bladder edema, hemorrhage, and urothelial damage (Korkmaz A 2007), whereas the pathophysiological role of cytokines, which are involved in PMN recruitment (TNF- α and IL-1 β), is also implicated in the progression of the disease (Hu VY 2003; Kiuchi H 2009; Smaldone MC 2009). The presence of the neurogenic component in HC is also thought to be due to increased concentrations of neuropeptides (CGRP and substance P) in the bladder tissue (Lucioni A 2008) together with an increased expression of TRPV1 mRNA (Dornelles FN 2014). Bladder barrier dysfunction was shown to be mediated through damage to nerve endings and spinal cord (L6 and S1) cells (Chuang YC 2009). The NK1-dependent PMN accumulation was also described in this model (Linares-Fernández BE 2007). Furthermore, tissue invasion of PMNs in the bladder could also be inhibited with TRPV1 antagonism (Dornelles FN 2014). It should be noted here that these experiments focused on the PMN–endothelial cell interactions in the early phase of the disease, when chemotherapy-related leukopenia cannot develop and the role of PMNs in the later phase of CYP-induced microcirculatory reactions can no longer be assumed.

Conclusions for Study 1

Based on our present results, it is not only IR (where direct/primary endothelial damage is present) that is linked to microcirculatory reactions which manifest in enhanced

PMN–endothelial interactions, but also HC and IC (where microcirculatory inflammatory reactions are most probably secondary after urothelial and interstitial damage). These observations suggest a potential role for microcirculatory reactions in the pathogenesis of these diseases.

5.2. Microcirculatory consequences of TRPV1-mediated acute neuropeptide release in the urinary bladder

TRPV1 agonism previously represented one of the possible treatment modalities for overactive bladder syndrome (Cruz F 1997; Fowler CJ 1992). The involvement of these afferent nerves in inflammatory processes of the urinary bladder have been demonstrated in both animal models (Birder LA 2018; Cruz F 1997) and human diseases (e.g. IC) (Andersson KE 2019; Liu L 2007). The cellular inflammatory processes and their dynamics associated with the topical administration of TRPV1 agonists, such as capsaicin and resiniferatoxin, have been investigated in the bladder here. This study was the first to demonstrate the dynamics of PMN–endothelial interactions following TRPV1 receptor activation evoked by capsaicin in the bladder. An early increase in the frequency of PMN–endothelial interactions (both rolling and adhesion) was observed in the bladder postcapillary venules as early as 15 min after the administration of capsaicin, and these inflammatory changes persisted throughout the entire experimental period (45 min). The early onset of inflammatory changes following capsaicin administration is consistent with previous observations that found a rapid development of NK1 receptor-dependent edema formation and PMN deposition after capsaicin in the skin (Boros K 2016) and pancreas (Grady EF 2000), and an even more rapid response occurring within minutes following an intratracheal administration of capsaicin (Baluk P 1995).

We have also demonstrated that the microvascular changes were brought about via a specific action of capsaicin on sensory nerves expressing TRPV1 receptors, since capsaicin-induced cellular inflammatory reactions were strongly inhibited by both TRPV1 receptor antagonism and the elimination of TRPV1-expressing nociceptive primary sensory neurons by neonatal capsaicin treatment. These observations suggest a neurogenic origin of the inflammatory process developing in the urinary bladder after the topical administration of capsaicin. The present findings also show that the effects of capsaicin are indirect and brought about by the release of pro-inflammatory neuropeptides known to be

released from activated TRPV1-expressing sensory nerves (Cruz F 1997; Szallasi A 1999). Since substance P and CGRP are the most abundant vasoactive peptides expressed in intact primary sensory neurons, we focused on the contribution of these neuropeptides to the capsaicin-induced microcirculatory reactions. The findings indicate that both CGRP and substance P contribute to the capsaicin-induced endothelial adhesion of PMNs, but rolling is only initiated by CGRP. It is reasonable to assume, therefore, that TRPV1-induced CGRP release initiates the PMN–endothelial interaction by promoting leukocyte rolling, but adhesion is influenced by both peptides. A similar conclusion was drawn in another study, which showed that substance P potentiated neutrophil accumulation in the skin only when it was released at a site where an inflammatory process had already been initiated (Cao T 2000).

In the urinary bladder, the capsaicin-induced expression of endothelial adhesion molecules manifested in the expression of E-selectin and ICAM-1 immunoreactivities in the postcapillary venules of the urinary bladder occurring in parallel with the cellular microvascular changes. Previous studies on the human cutaneous vascular bed also demonstrated a very rapid PMN accumulation (in approximately 15 min) after intradermal injection of substance P or CGRP (Smith CH 1993), and this is linked to an increased expression of ICAM-1 mRNA (within 60 min after substance P exposure) (Quinlan KL 1998). Enhanced ICAM-1 and E-selectin protein expressions develop over a longer time frame (these were typically examined for four hours or more after the topical application of substance P on the skin) (Lindsey KQ 2000; Quinlan KL 1998; Smith CH 1993). As for the urinary bladder, the NK1 receptor-mediated expression of ICAM-1 occurred as early as an hour after a local arterial injection of substance P (Chien CT 2003). Another potential mechanism of substance P on leukocyte accumulation is an NK1-independent pathway via mast cell activation which has been implicated in the skin in animal models (Saban MR 1997; Walsh DT 1995) and in humans as well (Okabe T 2000, 2001).

Still, little is known about the subcellular regulatory mechanism by which TRPV-related neuropeptide release modulates these changes, and the pathway by which capsaicin achieved these effects remained basically unexplored. The remarkably rapid (less than 60 min) increase in the expression of endothelium-derived adhesion molecules demonstrated here probably cannot be explained by the most time-consuming Ca^{2+} -dependent activation of NF- κ B pathways (Lindsey KQ 2000). The involvement of mechanisms related to a

capsaicin-induced intracellular free radical production, however, cannot be ruled out (Chien CT 2003). Clearly, the mechanistic background of the particularly rapid adhesion molecule expression in the rat urinary bladder warrants a further in-depth investigation.

The present findings may also be of relevance to neurogenic inflammatory processes that develop in other organs under particular pathological conditions. The dynamics and extent of the inflammatory process may depend not only on the sensory innervation density and the distribution of TRPV1 receptors, but also on other factors, such as the density of CGRP and substance P receptors and the adhesion molecule expression of the particular tissue/organ.

The TRPV1-related CGRP release was also shown to modulate immunological reactions, such as antigen presentation (Asahina A 1995), macrophage function (Ichinose M 1996), and cytokine production (Monneret G 2000). Even sepsis-induced cellular (macrophage-related) and vascular reactions (hypotension) are modulated by TRPV1 (Monneret G 2000; Wang Y 2008), in which sensing of sepsis-induced acidification via this receptor can also play a role (Bátkai S 2004; Varga K 1998). The protective role of TRPV1 has been demonstrated in endotoxemia and sepsis (Clark N 2007; Helyes Z 2007). TRPV1 also plays a protective role against postischemic reperfusion injury in the heart, possibly via substance P release from sensory nerve terminals (Wang L 2005).

Lastly, the potential clinical relevance of the present study should be noted, since TRPV1-expressing bladder sensory nerves used to serve as therapeutic targets in the treatment of overactive bladder syndrome and related disorders of the urinary bladder, such as IC. Quite significantly, in patients with idiopathic detrusor overactivity, TRPV1 agonist vanilloid compounds, such as resiniferatoxin and capsaicin, were only effective in patients that display an overexpression of the TRPV1 receptor in the urinary bladder (Kissin I 2011; Liu L 2007).

Conclusions for Study 2

The present findings suggest that the activation of TRPV1 receptor-expressing nociceptive afferent nerves in the urinary bladder triggers a coordinated inflammatory process involving arteriolar vasodilatation and plasma extravasation as well as an increase in PMN–endothelial interactions which lead to the transmigration of PMNs. These capsaicin-

induced local microcirculatory inflammatory side-effects in the urinary bladder are related to PMNs and are mediated via a TRPV1 receptor-dependent mechanism. The resultant increase in PMN rolling can be inhibited by CGRP receptor antagonism, whereas PMN adhesion can be ameliorated by both CGRP and NK1 receptor antagonism.

5.3. Suggested sequence of diagnostic interventions aiding assessment of severity and urodynamic features of the two major clinical manifestations of neurogenic bladder. Therapeutic considerations

Differential diagnosis difficulties with neurogenic bladder arise from the fact that many diseases cause symptoms similar to those of neurogenic bladder and findings from basic laboratory examinations, e.g. urinary retention, urinary reflux, pyelectasia, or pyuria, can be observed in numerous diseases without any neurogenic component. The diagnostic algorithm outlined here can aid in determining the location of the lesion (i.e. with neurological examinations) and clarifying whether we are facing an “overactive or flaccid type” of neurogenic bladder (using urodynamic examinations). Test results can range from minimal urinary retention to the most severe stage of hydronephrosis.

During diagnosis, the first step is to differentiate between overactive (Type #1) and underactive (Type #2) forms of urinary bladder. Anamnesis, routine urinalysis, and even routine ultrasound do not lead to the separation of the two types (since the last of these three only confirms the fact of urinary retention in both types); therefore, invasive urodynamic examinations are required to detect dynamic, functional changes in urination and so that the severity of these alterations can also be clarified. Therefore, urodynamic examinations (cystometry and uroflowmetry) (Amarenco G 2017; Cameron AP 2016; Schäfer W 2002;) supplemented with neurological (Podnar S 2001) and electromyographic examinations (Allert M 1973; Bacsu CD 2012) are indispensable in differentiating between the two major forms of neurogenic bladder. The primary aim of the proposed algorithm is not only to differentiate between the two major types of neurogenic bladder, but also to facilitate assessment of the severity and staging of related functional and morphological alterations and to aid in differential diagnosis.

Differential diagnostic considerations

During differential diagnosis, finding the cause of urinary infection is the first step because “simple urinary infection” causes symptoms similar to that of the overactive form of neurogenic bladder, including frequent urination and pyuria (the latter being confirmed by standard and microbiological examination of the urine), but incontinence is not present in these cases. In the case of simple urinary infection, kidney and bladder ultrasound will not detect urine retention but will certainly show signs of neurogenic bladder-related retention, i.e. morphological changes in upper urinary tract dilatation (i.e. pyelectasy). Furthermore, standard antibiotic therapy usually brings about relatively rapid amelioration of the symptoms of simple urinary infections.

In terms of the hyperactive form of neurogenic bladder, IC, HC, and bladder tumors should be excluded. In the case of IC, the symptoms are similar to Type #1 (hyperactive) neurogenic bladder with frequent urination, but anamnestic data also reveal typical urethral pain in the former case. Furthermore, cystoscopy confirms hyperemic areas with spiral vessels and Hunner’s lesions (in severe cases). HC can be learned from anamnestic data. Urinary bladder tumors also often cause hyperactive bladder-like symptoms, with frequent and urgent urination stimuli. A differential diagnosis is based on examination of urine sediment and cytological examinations, bladder ultrasound, and cystoscopy. For Type #2 (flaccid) urinary bladder, prostate hyperplasia and pelvic tumors can also cause similar storage/urination disorders, while urethral stricture and constipation may primarily cause urination problems. To differentiate them from Type #2 neurogenic bladder, anamnestic questionnaires, ultrasound scans, and uroflowmetry may help.

Therapeutic considerations

As noted above, the proposed diagnostic scheme may provide guidance to establish diagnosis and differential diagnosis of neurogenic bladder, and importantly, supplies information on the severity of the urodynamic and morphological alterations (Hinkel A 2007). This is particularly important because in most cases of neurogenic bladder, symptom relief cannot be achieved by treating the underlying cause; therefore, it is typically treated with approaches used to treat other diseases showing similar symptoms (thus independently of the cause). The main goal of the therapy is to prevent upper urinary tract damage while reducing the patients’ subjective symptoms (by reducing urgent urination and improving

continence). These test results serve to select the appropriate and optimal (often individualized) therapy while minimizing potential complications.

Among possible therapies of the overactive form of neurogenic bladder, relaxation of the detrusor muscle can be achieved with antimuscarinic agents (as a primary choice) because of their relatively good tolerability and acceptable side-effects (Stöhrer M 2007). Although antimuscarinic agents may improve storage symptoms, they may also lead to retention, which can be prevented by alpha adrenergic receptor antagonists in a combination therapy (Burkhard FC 2020). Bladder compliance can also be improved with the tricyclic antidepressant imipramine in combination with the agents noted above (Chancellor MB 1994). Relaxation of the detrusor muscle can also be brought about with beta adrenergic agonists (Nitti VW 2013). Another solution is the inhibition of urine excretion via the vasopressin pathway (Szollar S 1995). Cannabinoids and selective serotonin–noradrenaline reuptake inhibitors may also be considered for the alleviation of symptoms. Modulation of the sensory part of the urination reflex (using capsaicin and resiniferatoxin) is less targeted nowadays because this approach can only succeed when detrusor overactivity is accompanied by an overexpression of TRPV1 in the urothelium and suburothelium (Kuo HC 2006; Liu HT 2007; Silva C 2002). In drug-resistant cases, blockade of the motoric function of the detrusor muscle is feasible with topical application of botulinum toxin (Majoros A 2020). In the case of flaccid bladder, relaxation of the bladder outlet (at the base of the bladder and the urethra) via inhibition of alpha adrenergic receptors can alleviate a weak urine stream and straining (which occur due to sphincter overactivity or detrusor hypofunction) (Burkhard FC 2020). Owing to a well-tailored therapeutic pharmacological regimen, symptoms can be reduced in approximately 60% of cases (Stöhrer M 2007). Invasive therapies (e.g. neuromodulatory, sling plastics, urine deviation, artificial sphincters, urethrotomy, and bladder augmentation) can also be considered if pharmacological treatments are ineffective or in the case of serious side-effects (Burkhard FC 2020). Unfortunately, catheterization/self-catheterization represents the only choice to protect the upper urinary tract in many cases of neurogenic bladder.

Conclusions for Study 3

In summary, the proposed algorithm may facilitate optimization of urological assessment by increasing its efficiency, focusing on examinations that are the most essential for the

diagnosis. These test results aid in selecting the most appropriate and optimal (often individualized) therapy, while minimizing potential complications. Therefore, this algorithm may be particularly useful for colleagues interested in neuro-urology (neurologists, urologists, and rehabilitation specialists) during their training in Hungary.

6. SUMMARY OF NEW FINDINGS

1. IC and HC are not linked to direct endothelial damage, but characterized by PMN–endothelial interactions within the bladder, similar to microcirculatory inflammatory complications observed in IR, where endothelial damage is a primary pathophysiological event. This observation suggests a potential general role of microcirculatory reactions, uniformly, in the pathogenesis of these diseases.
2. Local capsaicin treatment induces rapid increases in PMN rolling and adhesion and expression of adhesion molecules E-selectin and ICAM-1 in the postcapillary venules of the urinary bladder. These changes can effectively be prevented by sensory chemodenervation with capsaicin and by competitive TRPV1 antagonism. The effect of specific receptor antagonists suggests that TRPV1-induced CGRP release initiates the PMN–endothelial interaction by promoting PMN rolling, but adhesion is mediated by both CGRP and substance P.
3. Based on available literature data and our experience, we propose an algorithm by which diagnosis and differential diagnosis of neurogenic bladder can be conducted in a timely and efficient manner. This may facilitate initiation of optimally tailored and individualized symptomatic treatment of neurogenic bladder with simultaneous minimization of the potential complications in the upper urinary tract.

7. ACKNOWLEDGEMENTS

I would like to express my gratitude to the Director of the Institute of Surgical Research, Prof. Mihály Boros, for making it possible to carry out my research at his institute. I wish to thank him for reviewing and correcting my publications and improving them with his suggestions.

I am grateful to Dr. Andrea Szabó for her guidance in designing and performing the experiments, writing papers and lectures on my research, and preparing the present thesis. Under her leadership, I mastered the basics needed to do scientific research, including conducting experiments, and to write scientific articles.

Special thanks go to Prof. Gábor Jancsó and Dr. Péter Sántha at the Department of Physiology for their scientific support and their contribution to the immunohistochemistry studies.

In addition, I wish to express my gratitude to all my co-workers and collaborators for their contributions to the studies, as well as to all the technicians at the Institute of Surgical Research for providing the conditions necessary for the experiments and the biochemical assays.

I would also like to thank my clinical supervisor, Dr. Ferenc Papp, and my colleagues at the Urology Department at the Bács-Kiskun County Hospital for supporting my research. Thanks as well to my family for their encouragement of my research and publications.

The studies reported in this thesis were made possible by Hungarian Scientific Research Fund grants OTKA 104656 and OTKA 109388, GINOP-2.3.2-15-2016-00015 and GINOP-2.3.2-15-2016-00034 grants, a National Research, Development and Innovation Office grant (NKFIH K116689), an EFOP-3.6.2-16-2017-00006 grant, and an NKFIH-1279-2/2020 grant.

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9. ANNEX

PAPER 1

A húgyhólyag gyulladásos mikrokeringési reakcióinak kísérletes vizsgálata

Experimental studies on microcirculatory inflammatory reactions of the urinary bladder

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Bevezetés: Átmeneti ischaemia kapcsán az ischaemia/reperfúziós (IR) károsodás elsődleges célpontja az erek endothelioma, míg interstitialis cystitisben és haemorrhagiás cystitisben a gyulladás az epithelium/urothelium felől indul, és az interstitium felé terjedhet, így okozva másodlagos mikrokeringési változásokat. Vizsgálataink célja a nem fertőzőes eredetű cystitisek és a hólyag IR mikrokeringési következményeinek vizsgálata, valamint a keringési reakciók összehasonlító elemzése volt. **Módszerek:** Hím Sprague Dawley-patkányokban protamin-szulfát (2 mg / 200 µl sóoldatban, $n = 6$) 30 perces intravesicalis instillációjával gyulladást indukáltunk, a második csoportban ($n = 5$) ciklofoszfamidot (75 mg/kg, i.p.) alkalmaztunk 24 órával a vizsgálat előtt. A harmadik csoportban ($n = 5$) a húgyhólyag átmeneti ischaemiáját idéztük elő a hólyagot ellátó erek 60 perces lezárásával. A kezeletlen kontrollcsoportban a húgyhólyagot fiziológiás sóoldattal instilláltuk ($n = 5$). A gyulladásos mikrokeringési reakciókat intravitális fluoreszcens videomikroszkóppal vizsgáltuk 60 perc reperfúzió után, illetve 24 órával a protamin-szulfát-instillációt vagy ciklofoszfamid-kezelést követően. **Eredmények:** A gördülő leukocyták aránya háromszorosára növekedett a hólyag posztkapilláris ereiben a protamin-szulfát-modellben, a ciklofoszfamid-csoportban ötszörös, az IR csoportban hat és félszeres volt a növekedés. A leukocyták kitapadása mindhárom kísérletes csoportban hasonló mértékben (közel hétszeresére) fokozódott. A vörösvértestek áramlási sebessége a protamin-szulfát- és IR csoportban csökkent, míg a ciklofoszfamid-csoportban kismértékben emelkedett. **Következtetések:** Eredményeink alapján nemcsak a direkt endotheliumsérülést okozó IR, de a protamin-szulfát és a ciklofoszfamid alkalmazása is mikrokeringési változásokat idéz elő a patkány húgyhólyagában. Ezek az eredmények felvetik a mikrokeringési zavarok kóroki szerepét az irritábilis hólyag szindróma és a haemorrhagiás cystitis pathogenesisében is.

Kulcsszavak: húgyhólyag-mikrokeringés, intravitális mikroszkópia, interstitialis cystitis, haemorrhagiás cystitis

Background: The vascular endothelium is a primary target of ischemia/reperfusion (IR) injury of the urinary bladder. In case of interstitial cystitis (painful bladder syndrome) or in cyclophosphamide-induced hemorrhagic cystitis, the injury is initiated at the epithelial/urothelial surface and propagates towards the interstitium, causing secondary involvement of the microvasculature. Hence the aim of our study was to assess and compare the microcirculatory aspects of the non-infectious forms of cystitis with that of IR-caused reactions. **Materials and Methods:** In male Sprague-Dawley rats, interstitial cystitis was induced by intravesical instillation of protamine sulphate (2 mg in 200 µl saline for 30 min; $n = 6$). In another group, cyclophosphamide (75 mg/kg, ip) was administered 24 hr prior to the experiments ($n = 5$). In the third group, urinary bladder ischemia was induced by 60-min occlusion of the vessels supplying the bladder ($n = 5$). The microcirculatory inflammatory reactions were investigated by fluorescence intravital microscopy 60 min after reperfusion and 24 hr after protamine sulphate instillation or cyclophosphamide administration, respectively. In the control group, the bladder was instilled with saline ($n = 5$). **Results:** Rolling of leukocytes increased ~3-fold in the postcapillary vessels in the protamine sulphate-treated group and the increase in this parameter was ~5 and ~6.5-fold in cyclophosphamide and IR groups, respectively. The increase in leukocyte adherence reached similar, approx. 7-fold increase in each of the challenged groups. The red blood cell velocity in the capillaries decreased in the protamine sulphate and IR groups, while the velocity increased moderately in the cyclophosphamide-treated group. **Conclusions:** Our results dem-

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Támogatás: OTKA K75161; TÁMOP-4.2.1/B-09/KONV-2010-0005

onstrate that direct endothelial injury (caused by IR), as well as protamine sulphate and cyclophosphamide administrations induce inflammatory microcirculatory changes of the urinary bladder. These observations suggest a causative role for microcirculatory disturbances in the pathogenesis of interstitial cystitis and hemorrhagic cystitis as well.

Keywords: urinary bladder microcirculation, intravital microscopy, interstitial cystitis, haemorrhagic cystitis

Beérkezett: 2012. június 27.

Bevezetés

A gyulladás minden lényegi eleme (*rubor, tumor, dolor, calor*) a microcirculatio szintjén zajló jelenségekre vezethető vissza. A mikrokeringés juttatja el az inflammatiós jeleket közvetítő molekulákat és sejteket az érintett szövetekhez, és e funkció ellátása során a mikroereket határoló endothel sejtek működése is megváltozhat. Az endothelium aktivációja az antigénekre adott „klasszikus” gyulladásos válasz során azonban nem iniciáló, hanem következményes tényező. Ugyanakkor más esetekben, így például az oxidatív károsodással járó hypoxia-reoxigenizációs folyamatokban az endothel réteg tekinthető a károsodás fő célszervének. A kimenet mindkét esetben hasonló lesz, végeredményképpen endothel aktiváció és/vagy az endothel sejtek dysfunctiója jelentkezik. Ennek következtében az endothelből származó vasoactiv mediatorok felszabadulása módosul, fokozódik a gyulladás sejtes fázisában szerepet játszó adhaesiós molekulák expressziója,^{1,2} és e folyamat részeként a sejt-sejt közötti reakciók (köztük a leukocytá-endothel sejt interakciók) véráramlási zavarokat okoznak. Az endothel dysfunctio létrejöttében főleg szabad gyökök által mediált reakciók feltételezhetők, de a reperfusio során aktiválódó proteolitikus enzimek (pl. elasztáz, cathepsin G, proteáz III) felszabadulásának is nagy jelentőséget tulajdonítanak.³ Ismert az is, hogy a mikrokeringés szintjén a sejt-gyulladásos reakciók a vérlemezkék és a polimorf magvú leukocyták fokozott kitapadásával, szöveti migrációval és további lokális reakciókkal járhatnak együtt.⁴

Az interstitialis cystitistről vagy más néven irritábilis hólyag szindrómáról (IHS) jól ismert, hogy a károsodás kezdetben az epithel réteget érinti. A klinikai jelekért és tünetekért a barrierkárosodás interstitialis komponense lesz a felelős,^{5,6} de a feltételezések szerint a kialakuló fekélyek hátterében a hypoperfúciónak is szerepe lehet.⁷ A húgyhólyag másik, gyakori, gyulladásos kórképe az alkiláló hatású kemoterápiás szerek iatrogén mellékhatásaként kialakuló haemorrhagiás cystitis (HC). A ciklofoszfamid toxikus metabolitja, az acrolein az urothelium felől vált ki gyulladásos reakciót, ami ezt követően hat az intestitiumra és a mikroerekre.⁸ Mindkét urológiai kórképben (IHS és HC) kimutatták a leukocyták szöveti infiltrációja mellett más, gyulladásos következményekkel járó tényezők (szenzoros neuron- és hízósejt-aktiváció) fontosságát,^{9–11} de a mikrokeringés szintjén létrejövő reakciók kórtani szerepe az IHS és HC pathogenesisében nem tisztázott.

Jelen kutatásunk során célul tűztük ki a húgyhólyag gyulladásos mikrokeringési reakcióinak összehasonlító

elemzését direkt, illetve indirekt endothel károsodással járó állapotok állatkísérletes modelljeiben. A közvetett endothel károsodással járó kórképek mikrokeringési manifesztációi kevésbé ismertek, és a tanulmányozásukra alkalmazott modellekben megfigyelhető mikrokeringési reakciókról sincs még információ.

A húgyhólyag-endothelium direkt károsodásával járó hypoxia-reoxigenizációs klinikai kórképek közé többek között az embolisatio, thrombophlebitis, vasospasmus, arteriosclerosis, terhesség, kismencedei tumorok, műtétek során alkalmazott érlektetés és trauma tartozik.¹² A hypoxia-reoxigenizáció következtében morfológiai és funkcionális károsodás alakul ki, ami a hólyagtágulékonyosság (compliance), -kapacitás és összehúzódnási képesség csökkenésében, a vér-vizelet gát károsodásában, oedemában, simaizom-degenerációban, necrosisban vagy apoptosiban nyilvánulhat meg.⁴ Ebben az esetben a mikrokeringési változások és a következményes szervfunkciós zavar közötti ok-okozati kapcsolat már viszonylag jól tisztázott, ezért jelen kísérleteink során a hólyag ischaemia-reperfusió (IR) modelljét összehasonlítás alapként alkalmaztuk a mikrokeringési zavar súlyosságának megítéléséhez.

Anyag és módszer

Kísérleteinket a NIH irányelvei alapján (Guide for the Care and Use of Laboratory Animals) végeztük a Szegedi Tudományegyetem Munkahelyi Állatkísérleti Bizottsága jóváhagyásával.

Sebészi beavatkozás

Hím Sprague Dawley-patkányokon (átlagos súly: 300 ± 20 g) intraperitonealisan adott Na-pentobarbitál anaestheziát (45 mg/kg) alkalmaztunk, szükség esetén további kis, fenntartó dózisokat adtunk intravénásan. A jobb oldali vena jugularist és arteria carotist kanüláltuk infúzió és gyógyszerek adása, valamint artériás vérnyomásmérés (Statham P23Db transzducer) céljából. Az állatokat fűtőpadra helyeztük, testhőmérsékletüket 36–37 °C között tartottuk, folyadékpótlásként 10 ml/kg/h Ringer-laktát-infúziót adtunk. A légzés biztosítása céljából a tracheát kanüláltuk. A húgyhólyagot medialis laparotomiából feltártuk, majd a kupolánál kanült vezettünk be az állandó intravesicalis nyomás fenntartása céljából, melyet 0,9%-os fiziológiás sóoldat szükség szerinti injektálásával értünk el.

Kísérleti protokoll

Az első sorozatban a húgyhólyag-ischaemiát (IR; $n = 5$) az aa. vesicales reverzibilis, 60 perces lekötésével érték el, majd további 30 perc után vizsgáltuk a mikrokeringési következményeket.⁴

A második sorozatban az IHS korai fázisát az urothelium szelektív károsítását okozó protamin-szulfát (2 mg / 200 μ l fiziológiás sóoldatban; $n = 6$) 30 perces intravesicalis instillációjával indukáltuk ketamin-xylazin altatásban, a hólyag kupolájának laparotomiát követő steril punkciója révén.¹³ A protamin-expozíciót követően a húgyhólyagot steril fiziológiás sóoldattal átmostuk, majd a hasat steril körülmények között két rétegben zártuk. Az indukciót követő 24 órával később vizsgáltuk a gyulladásos reakciókat.

A második sorozat következő, cystitis csoportjában, a HC kísérletes modelljében az állatokat ciklofoszfamid toxikus dózisával kezeltük (40 mg/kg i.p.; 0,1 ml / 100 g, $n = 5$). A mikrokeringési vizsgálatokat 24 óra elteltével végeztük.

Kontrollként az előbbi protokolloknak megfelelő álműtött állatok csoportját alkalmaztuk, amelynél a hólyagokat fiziológiás sóval instilláltuk (álműtött csoport, $n = 5$).

Intravitalis videomikroszkópia

A kipreparált húgyhólyagot speciális tartó segítségével kiemeltünk a hasüregből a szerv vérellátásának megtartása mellett, majd a vizsgálat idejére vékony műanyag fóliával fedtük a kiszáradás és a hővesztés minimalizálása céljából. A húgyhólyag mikrokeringését Zeiss AxioTech Vario 100HD intravitalis videomikroszkóppal vizsgáltuk (100 W

HBO higanygőz-lámpa, Acroplan 20 vízimmerziós objektív). Fluoreszcein-isothiocianáttal (Sigma Chemicals, St. Louis, MO) jelölt vörösvértestek (0,2 ml i.v.) szolgáltattak fluoreszcens perfúziós markerként (1.A ábra); a leukocyták festésére rhodamine 6G-t (Sigma Chem. 0,2%, 0,1 ml i.v.) használtunk (1.B ábra). A mikroszkópos képeket CCD videokamera (AVT HORN-BC 12) segítségével S-VHS videorekorderrel (Panasonic AG-MD 830) rögzítettük, amit személyi számítógéphez csatlakoztattunk az adatok analízise céljából.

Videoanalízis

A mikrokeringési paraméterek kvantitatív meghatározása a kísérleteket követően off-line történt a videofelvételek frame-to-frame analízisével, szoftver (IVM, Pictron Ltd., Budapest) segítségével. A vörösvértestek áramlási sebességét (RCV; μ m/s) 5 különböző látótérben és 5 kapillárisban mértük a kísérlet minden mérési időpontjában (25 mérés átlagát számoltuk). A leukocytá-endothel sejt interakciókat állatonként 5 posztkapilláris venulában vizsgáltuk, melyek átmérője 10–20 μ m között volt. Kikapadó leukocytáknak azokat a sejteket definiáltuk, melyek nem mozdultak, vagy legalább 30 másodpercig rögzültek az endothel sejt felszínéhez a megfigyelési periódus alatt, számukat 1 mm² endothelium felszínre vonatkoztattuk. Gördülő leukocytáknak azokat a sejteket tekintettük, melyek sebessége nem érte el a mikroerek középvezetékében mért vörösvértest áramlási sebesség 40%-át. Számukat egységnyi idő alatt egy adott érkeresztmetszeten áthaladó, gördülést mutató sejtek számaként adtuk meg.

1. ábra. Reprezentatív intravitalis videomikroszkópos felvételek a húgyhólyag mikrokeringéséről a vörösvértestek (A) és a neutrophil leukocyták (B) jelzése mellett. A fehér vonal 50 μ m-t jelez

Statisztikai analízis

Az adatok analízise SigmaStat for Windows (Jandel Scientific, Erkrath, Németország) szoftverrel történt. A csoportok közötti különbségek kimutatásához egyutas ANOVA-t használtunk, melyet Bonferroni *post hoc* teszt követett. Statisztikailag szignifikánsnak $p < 0,05$ esetén tekintettük a különbségeket.

Eredmények

A húgyhólyag-endothelium direkt károsodásával járó IR hatására a leukocyta-rolling mintegy hétszerese volt az álműtött csoport értékeinek (2. ábra). A protamin-szulfát-instillációt követően 24 órával a húgyhólyag posztkapilláris venuláiban mintegy háromszoros, a ciklofoszfamid-kezelés után pedig közel ötszörös leukocytarolling-értékeket találtunk az álműtött (fiziológias sóoldattal instillált) csoport értékéhez képest (2. ábra). A leukocyta-kitapadás mind-

2. ábra. Leukocyta-rolling a húgyhólyag posztkapilláris venuláiban álműtött, protamin-szulfáttal (PS) vagy ciklofoszfamiddal (CYP) kezelt és hólyag ischaemia-reperfúzió (IR) alávetett állatokban. Átlag $\pm$ SEM. * $p < 0,05$ vs intakt álműtött állatok

3. ábra. A leukocyták kitapadása a húgyhólyag posztkapilláris venuláiban álműtött, protamin-szulfáttal (PS) vagy ciklofoszfamiddal (CYP) kezelt és hólyag ischaemia-reperfúzió (IR) alávetett állatokban. Átlag $\pm$ SEM. * $p < 0,05$ vs intakt álműtött állatok

három modellben hasonló mértékben változott, mintegy hétszeresére fokozódott az álműtött állatok értékeihez viszonyítva (3. ábra).

A kapillárisok vörösvérsejt áramlási sebessége csak az IR után változott szignifikánsan, mintegy 30%-kal alacsonyabb volt az álműtött csoport értékeihez képest (4. ábra). A protamin-szulfát-kezelés után (IHS modell) is csökkent áramlási sebességértékeket találtunk, azonban az eltérés mértéke (mintegy 15%) nem volt szignifikáns. A ciklofoszfamid-kezelés után (HC modell) azonban kissé (de nem szignifikánsan) magasabb vörösvérsejt áramlási sebességeket detektáltunk a kapillárisokban.

Megbeszélés

Mai ismereteink szerint az IR funkcionális károsodást okoz a húgyhólyagban, melynek egyik potenciális kóroki tényezője a mikrokeringés zavara. Az ok-okozati összefüggés kísérletesen igazolható, ugyanis az ischaemiás prekondicionálás nemcsak a posztischaemiás mikrokeringési változásokat mérsékli, de kivédi a kontraktilitási zavart is.³ Más modellekben, így például az indirekt endotheliumkárosodással járó IHS vagy HC eseteiben ez az összefüggés azonban még nem tisztázott.

Kísérleteink célja a mikrokeringés szintjén megvalósuló reakciók megismerése volt olyan modellekben, ahol az endothelialis aktiváció/károsodás nem kóroki, hanem következményes jelenség. Az IHS etiológiája nem pontosan ismert, és számos teória látott napvilágot, így infekció, toxikus metabolitok a hólyagban, urothelialis dysfunctio és neurogén hatások¹⁴⁻¹⁶ szerepe is felmerült. Proinflammatorikus citokinek és chemokinek fokozott felszabadulását és receptoraik gátlásának kedvező hatását is leírták már,^{17,18} így ezek a megfigyelések a gyulladásos reakciók jelentőségére utalnak. Általánosságban elmondható, hogy a gyulladásos citokinek nemcsak az immunológiai reakciókat modulálják, de a szenzoros folyamatokat is, melyek közvetlen

4. ábra. A vörösvértestek áramlási sebessége a húgyhólyag kapillárisaiban álműtött, protamin-szulfáttal (PS) vagy ciklofoszfamiddal (CYP) kezelt és hólyag ischaemia-reperfúzió (IR) alávetett állatokban. Átlag $\pm$ SEM. * $p < 0,05$ vs intakt álműtött állatok

módon hatással lehetnek a vizeletürítés szabályozására, azaz a hólyag alapfunkciójára is.¹⁹ Ezt a gondolatmenetet támasztják alá azok a klinikai megfigyelések is, amelyek fokozott gyulladásos infiltrációról számolnak be IHS-betegeknél.¹⁶ Fokozott kemotaktikus kemokinreceptor-szignalizáció mutatható ki az általunk vizsgált másik klinikai kórkép, a HC modelljében is, ennek az útvonalnak a gátlása mérsékelte a hólyag-hyperexcitációt, és a compliance-t is javította patkányon.²⁰

A mikrokeringésben manifesztálódó hólyaggyulladás vizsgálatakor az IR károsodást viszonyítási alapként használtuk. Korábban ebben az állapotban kimutatták, hogy a microperfusio és microvascularis permeabilitas zavara együtt jár a sejt-sejt szintű gyulladásos reakciók fokozódásával.⁴ A perfusio során kialakuló fehérvérsejtes beszűrődésért felelős sejt-felszíni receptorok jól ismertek,^{13,21} és a leukocyták által kiváltott károsodást a kitapadást gátló antitestekkel kedvezően lehetett befolyásolni.²² Ismert az is, hogy a húgyutak urotheliuma és az izomszövet mellett az endothel sejteken elhelyezkedő vasoconstrictiv endothelin receptoroknak is kifejezett szerepük van a hólyag IR által kiváltott mikrokeringési változásokban.²³ A hólyag esetében a perfusio jellegzetessége a hypoperfusio, vagyis ebben a szervben nem jelentkezhethet a klasszikus *rubor* (ami a mikrokeringés szintjén a hyperaemiának felel meg). A csökkent áramlási sebesség magyarázata az endothelium által termelt, vasoconstrictio irányában ható mediatorok felszabadulásának túlsúlyában és/vagy a direkt endotheliumpkárosodásban, valamint a perfusio során létrejövő fokozott oedemaképzésben (fokozott microvascularis károsodás) együttesen kereshető.⁴

Az IHS folyamatának korai fázisa jól modellezhető az urothelium acut, szelektív károsítását okozó intravesicalis protamin-szulfáttal,⁷ mely a glükóz-aminoglikán-réteget reverzibilisen inaktiválja.²⁴ A protamin-szulfát által kiváltott gyulladásos reakciók az epithelium károsítása és a következményes permeabilitásfokozódás révén valósulnak meg.⁷ A leukocyta-invázióról és a hízósejtek aktivációjáról már korábban beszámoltak protamin-szulfát által okozott cystitis esetén,^{25,26} de a mikrokeringési zavart a jelen munka írja le elsőként. Kísérleti eredményeink azt igazolták, hogy a nem közvetlenül endotheliumpkárosodással járó kórképekben is az IR-hoz hasonló mikrokeringési zavar figyelhető meg. Ennek jeleit a sejt-sejt interakciók vonatkozásában figyelhettük meg mind az IHS, mind a HC modellje esetében. Az IHS pathogenesisében a mikrokeringési változások jelentőségét támasztják alá azok a megfigyelések, ahol a hólyagbarrier-funkció javítása enyhítette a klinikai tüneteket, és a részleges keringési zavarra utaló feké-

lyek száma is mérséklődött.⁷ Vannak olyan feltételezések is, melyek szerint az irritáló ágensek felszabadulása az át-eresztővé vált epitheliumon keresztül neurogén gyulladásos aktivációt okoz. Mások a nitrogén-monoxid (NO) relatív hiányát feltételezik a kórkép pathogenesisében, mivel az NO-donor L-arginin mérsékli a tüneteket.²⁷ A lokális szabadgyök-felszabadulás szintén feltételezhetően oki tényező, mivel a szabadgyök-fogó melatonin²⁸ és DMSO²⁹ is kedvezően befolyásolja a kórkép kimenetelét. A hízósejtek és a szenzoros neuronok közeli lokalizációját igazolták mind irritábilis bél szindrómában, mind IHS-ben.^{30–32}

A klinikumban a nem infekciós etiológiájú cystitisek közül leggyakrabban a ciklofoszfamid-indukált HC fordul elő. A cystitis e formája a legszélesebb körben alkalmazott állatkísérletes cystitismodell, mely az IHS-hez hasonló tüneteket okozva a vizeletürítési reflexek jelentős mértékű átalakulásával jár.³³ A HC-ciklofoszfamid cystitisben a neurogén komponens jelenlétét is feltételezik, mivel neuropeptidek (CGRP, substance P) fokozott koncentrációja mutatható ki a hólyagszövetben,³⁴ és a húgyhólyag barrier-funkciójának zavara az idegvégződés és a gerincvelő (L6, S1) sejtjeinek a károsodása révén valósul meg.³⁵

Jelen kísérleteinkben mind IHS, mind HC modellben a leukocyta-endothel sejt interakciók jelentős fokozódását észleltük. Más megfigyelések jelentős génexpressziós változásokat igazoltak a leukocytákban IHS-ben humán körülmények között,³⁶ fokozott leukocytaszámot mutattak ki biopsziás mintákból,⁵ illetve igazolták a leukocyta-endothel sejt interakciók egyik fontos endothelialis komponensének, az ICAM-1 adhaesiós molekulának a fokozott expresszióját.¹ Kísérletes IHS-ben leukocyta citokin ligandban bekövetkező változásokat találtak.⁹ Hasonlóképpen, a ciklofoszfamid által kiváltott cystitisben az oedema, ulceratio és haemorrhagia mellett a leukocyták fokozott infiltrációját is leírták.⁸ Meg kell itt jegyezni, hogy kísérleteinkben a leukocyta-endothel sejt interakciók *in vivo* vizsgálatát a kórkép korai fázisban végeztük, amikor a kemoterápia miatt még nem alakulhatott ki leukopenia, ugyanakkor a ciklofoszfamid-kezelés későbbi fázisában a leukocyták szerepe már nem feltételezhető.

Eredményeinket összefoglalva elmondhatjuk, hogy nemcsak a direkt endotheliumpkárosodással járó ischaemia-reperfusio, hanem az urothelium károsodása útján iniciálódó haemorrhagiás és interstitialis cystitis is együtt jár a húgyhólyag következményes, mikrokeringési gyulladásos reakcióival, melyek elsősorban a leukocyta-endothel sejt interakciók fokozódásában nyilvánulnak meg. Ez a felismerés felveti a mikrokeringési zavar kóroki szerepét a fenti kórképek pathogenesisében is.

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PAPER 2

Capsaicin-induced rapid neutrophil leukocyte activation in the rat urinary bladder microcirculatory bed

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Funding information

European Structural and Investment Funds, Grant numbers: GINOP-2.3.2-15-2016-00034, EFOP-3.6.2-16-2017-00006; National Research, Development and Innovation Office, Grant number: NKFIH K116689

Aims: This study was initiated to investigate the involvement of neutrophil leukocyte activation in neurogenic inflammation, a process also involved in human urinary pathologies, elicited in the rat urinary bladder by the local administration of capsaicin, the archetypal TRPV1 agonist. The contribution of afferent nerves and sensory neuropeptides to leukocyte activation in the urinary bladder microcirculatory bed was examined.

Methods: Following a 15-min topical application of capsaicin (50 μ M), leukocyte-endothelial interactions were examined for an observation period of 45 min with intravital microscopy. Expression of adhesion molecules E-selectin and ICAM-1 implicated in these interactions was assessed by immunohistochemistry. Selective sensory denervation was performed by neonatal treatment with capsaicin. The role of the TRPV1 receptor and two sensory neuropeptides (CGRP and substance P [SP]) were studied using the selective antagonists capsazepine, CGRP₈₋₃₇ and RP67580, respectively.

Results: Capsaicin induced rapid increases in leukocyte rolling and adhesion and increased the expression of E-selectin and ICAM-1 in the postcapillary venules. Sensory chemodenervation via capsaicin and also TRPV1 receptor antagonism effectively prevented these changes. A similar reduction was observed in leukocyte adhesion after topical application of CGRP₈₋₃₄ or RP67580, but only CGRP₈₋₃₄ reduced the capsaicin-evoked leukocyte rolling.

Conclusions: Topical application of capsaicin induces early neurogenically mediated cellular microcirculatory inflammatory reactions via the activation of the TRPV1 receptor and the release of CGRP and SP from sensory nerves in the bladder. Co-administration of SP and CGRP receptor antagonists may ameliorate microcirculatory inflammatory changes elicited by capsaicin in the urinary bladder.

KEYWORDS

capsaicin, CGRP, leukocyte rolling and adhesion, substance P

1 | INTRODUCTION

C-fiber chemosensitive primary sensory neurons innervate a variety of organs and tissues and express the transient receptor potential vanilloid type 1 (TRPV1) receptor.¹ The activation of the TRPV1 receptor by noxious stimuli results not only in the generation of nociceptive impulses transmitted to the central nervous system, but also in the release of neuropeptides from the stimulated sensory nerve endings. Neuropeptides released from sensory nerves initiate an array of local tissue reactions, including vasodilatation and plasma extravasation, that is, a neurogenic inflammatory response. There is ample evidence of the role of chemosensitive primary afferents in the transmission of nociceptive impulses and mediation of neurogenic inflammation in a variety of organs and species, including man.^{1–3} Studies on the innervation of the rat urinary bladder revealed that the organ is richly served by capsaicin-sensitive afferent nerves, the majority of which contain peptides.¹ This particular class of sensory nerves significantly contributes to the regulation of urinary bladder functions including the micturition reflexes in both animals in general and man.^{4,5} Moreover, several pathological conditions of the urinary bladder, including neurogenic detrusor overactivity, idiopathic detrusor overactivity, and bladder outlet obstruction involve changes in C-fiber afferent mechanisms.^{4–6} TRPV1 is involved in the transmission of urinary bladder pain and the application of TRPV1 agonists, and by eliminating capsaicin-sensitive sensory nerves, it has a therapeutic use in the treatment of pathological pain conditions of the urinary bladder. TRPV1 is also critically involved in inflammatory processes of the urinary bladder. In patients suffering from interstitial cystitis/bladder pain syndrome, the severity of the clinical symptoms and inflammation was correlated with the enhanced expression of TRPV1-immunoreactive nerve fibers in the urinary bladder.⁷ Hence, inflammatory reactions elicited through activation of the TRPV1 receptor by the local/intravesical application of vanilloid agonists, such as capsaicin or resiniferatoxin are of particular interest, as these agonists are used therapeutically to treat disorders such as neurogenic detrusor overactivity.^{5,8}

TRPV1 activation is known to bring about characteristic microcirculatory reactions in several organs. The activation of the TRPV1 receptor, through the release of vasoactive peptides such as calcitonin gene related peptide (CGRP) and substance P (SP) from the sensory nerves, results in an extremely rapid elevation in blood flow and an increase in cutaneous and dural permeability.^{9,10} TRPV1-mediated vasodilatory effects are partially linked to the release of CGRP in the dura mater and the skin.^{9,11} Another, indirect mechanism involves the enhanced activation of endothelial Ca^{2+} -dependent nitric oxide synthase and consequent nitric oxide release elicited through a TRPV1-dependent rise in

intracellular Ca^{2+} . This is supported by findings showing that the release of nitric oxide could be inhibited by pretreatment with the TRPV1 receptor antagonist capsazepine *in vitro*¹² and by sensory denervation *in vivo*.¹³ Edema formation elicited by the activation of TRPV1 receptors, however, is linked to the release of SP and its binding to endothelial neurokinin-1 (NK1) receptors that lead to an increase in vascular permeability and polymorphonuclear leukocyte (PMN) infiltration,¹⁰ where the dose-dependent increase in plasma extravasation evoked by SP and capsaicin could be inhibited by capsazepine^{10,14} or by NK1 receptor antagonism.³

Even though TRPV1 agonists are used in the therapeutic management of urinary bladder disorders, the potential acute microcirculatory side effects of TRPV1 activation have not yet been examined. Therefore, the present study was designed to examine, by using intravital videomicroscopy and immunohistochemistry, the effects of capsaicin, the archetypal TRPV1 agonist on PMN-endothelial cell interactions in the microcirculatory system of the rat urinary bladder. The contribution of sensory nerves and the sensory neuropeptides SP and CGRP to these inflammatory phenomena was studied with the aid of selective sensory chemodenervation and pharmacological antagonists.

2 | MATERIALS AND METHODS

The project was approved by the National Scientific Ethical Committee on Animal Experimentation (National Competent Authority), with the license number: V./144/2013. The study was performed in adherence with the EU Directive 2010/63/EU on the protection of animals used for experimental and other scientific purposes and the National Institute of Health guidelines for the use of experimental animals.

2.1 | Neonatal capsaicin treatment

Newborn male Sprague-Dawley rats were injected subcutaneously with a single dose of 50 mg kg^{-1} capsaicin or its vehicle (6% ethanol and 8% Tween 80 in saline) on day 2 of life under ether anesthesia. Neonatal capsaicin treatment has been shown to produce a selective degeneration of chemosensitive afferent nerves which are sensitive to capsaicin and express the TRPV1 receptor.^{1,15} The eye-wiping test, using a 1% solution of zingerone dripped into the eyes of rats on the day of the experiment, was used to demonstrate the elimination of TRPV1-expressing afferent nerves.¹⁵

2.2 | Surgical procedures

Healthy adult Sprague-Dawley rats (and the average weight was $320 \pm 10 \text{ g}$) were anesthetized intraperitoneally with an

initial dose of sodium pentobarbital (45 mg kg^{-1}) and placed in a supine position on a heating pad to maintain the body temperature at $36\text{--}37^\circ\text{C}$. The trachea, the carotid artery, and jugular vein were cannulated to facilitate spontaneous respiration, and for continuous measurement of blood pressure and the injection of fluorescent dyes, respectively. There were no significant differences in the blood pressure among the different experimental groups (see later) at any time-point of the microcirculation measurements (data not shown).

The urinary bladder was prepared for intravital microscopic (IVM) examination using a modified method of Bajory et al.¹⁶ Following a midline laparotomy, the bladder was exposed and the median umbilical ligament resected. A polyethylene catheter (ID 0.28 mm, OD 0.61 mm; Smiths Medical, UK) was later inserted into the bladder dome and fixed by a ligature (via a 3-0 braided polyglycolic acid thread). As the catheter insertion caused nearly complete emptying of the bladder, 0.5 mL of a body-warm 0.9% NaCl solution was injected to provide a similar, standard baseline filling state of the bladders in all of the experiments. In pilot studies, the resulting intravesical pressure ($<7 \text{ mm Hg}$) did to cause any microcirculatory disturbances in the bladder. The remaining abdomen was covered with a Saran wrap to avoid fluid and heat loss and drying out of the surface of the abdominal organs.

2.3 | Experimental protocol

The aim of our experiments was to examine the microcirculatory inflammatory consequences of a 15-min topical application of capsaicin on the bladder surface. We used two major sets (see Figure 1). In Set 1, the effects of TRPV1 receptor antagonism as well as the effects of ablation of TRPV1-positive nerves (achieved by the neonatal administration of capsaicin) were examined. In Set 2, the effects of selective CGRP and NK1 receptor antagonists were studied by monitoring the capsaicin-induced cellular inflammatory changes. All topical treatments were performed by placing pieces of Gelaspon[®] sponge (Chauvin Ankerpharm GmbH, Berlin, Germany) soaked in the respective compound(s) onto the anterior surface of the bladder for 15 min.

In the first set of experiments, the animals were allotted to one or the other of the following experimental groups: the anterior surface of the bladder was topically treated with capsaicin or its vehicle by dripping 100 μL of the solution on a Gelaspon sponge which was placed on the bladder dome for 15 min in the first two groups (vehicle and capsaicin groups, $n = 6$, and $n = 10$, respectively). The effect of the specific TRPV1 inhibitor capsazepine on the microcirculatory changes in two groups where capsazepine was topically applied just before the administration of capsaicin or its vehicle (vehicle + capsazepine and capsaicin + capsazepine group, $n = 6\text{--}6$). These findings

were compared with those obtained in rats ($n = 6$) treated with capsaicin as neonates.¹⁵ In the second set of experiments, the effects of the capsaicin receptor antagonist capsazepine were studied on the capsaicin-induced inflammatory changes. During these experiments (the second set), the microcirculatory effect of the CGRP and NK1 antagonism was examined and CGRP₈₋₃₇ (10 μM) and RP67580 (10 μM) were applied on the bladder surface in the presence (capsaicin + CGRP₈₋₃₇; $n = 5$) and capsaicin + RP67580; $n = 7$) and in the absence of topical capsaicin (vehicle + CGRP₈₋₃₇; $n = 6$), and vehicle + RP67580; $n = 8$) (50 μM). After a 15-min exposure, the sponge was removed from the bladder surface and thoroughly rinsed off with warm physiological saline and covered with a Saran wrap for the remainder of the duration. IVM recordings were made 15, 30, and 45 min after topical treatments. This overall 60-min observation period was chosen because accumulation of the fluorescence tracers in the urine with resulting deterioration in the contrast of the images did not permit a longer observation period.

2.4 | Intravital microscopy

The anterior surface of the bladder microcirculation was visualized by IVM (Zeiss Axiotech Vario 100HD microscope; Carl Zeiss GmbH, Jena, Germany; 100-W HBO mercury lamp; Acroplan 20 $\times$ water immersion objective). The animals received intravenous injections of fluorescein isothiocyanate (FITC)-labeled erythrocytes¹⁷ (0.2 mL; Sigma, St. Louis, MO) for the verification of the intact perfusion of the examined microvascular bed (using the 490/525 nm filter set of the microscope). Leukocytes were stained by injecting rhodamine 6G intravenously (0.2%, 0.1 mL; Sigma) and examined by a 526/555 nm filter set (Figure 2). Images were recorded with a charge-coupled device video camera (Teli CS8320Bi, Toshiba Teli Corporation, Osaka, Japan) attached to an S-VHS video-recorder (Panasonic AG-MD 830; Matsushita Electric Industrial Co., Tokyo, Japan) and a personal computer.

2.5 | Analysis of microcirculation data

A quantitative evaluation of the microcirculatory parameters was performed off-line by a frame-to-frame analysis of the videotaped images taken for IVM (IVM Software; Pictron Ltd, Budapest, Hungary). Leukocyte-endothelial cell interactions were analyzed in four or more postcapillary venules within a minimum of three observation fields per rat (choosing the most representative vessels). All of the evaluations were performed by two investigators. Rolling leukocytes were defined as cells moving with a velocity less than 40% of that of the erythrocytes in the centerline of the microvessel and passing through the observed vessel segment being observed within 30 s, and are expressed as the number of cells per mm per sec.¹⁸ Adherent

FIGURE 1 The time sequence of treatments and measurements in experimental groups in Sets 1 and 2. The topical treatments with capsaicin (CAP, 50 μ M) or other compounds (see below) were performed at the anterior surfaces of the bladders for 15 min. In Set 1, the TRPV1 receptor antagonist capsazepine (CZP, 200 μ M topically) and ablation of the TRPV1-positive nerves (achieved by the neonatal administration of CAP) were applied. In Set 2, the consequences of selective CGRP and NK1 receptor antagonism were investigated by topically applying the selective CGRP receptor antagonist CGRP₈₋₃₇ (10 μ M) and the NK1 receptor antagonists RP67580 (10 μ M) together with CAP. Here, IVM = intravital microscopic examinations of leukocyte-endothelial interactions in postcapillary venules of the bladder

leukocytes were defined as cells that did not move or detach from the endothelial lining within an observation period of 30 s and are expressed as the number of cells per mm^2 of endothelial surface, calculated using the diameter and length of the vessel segment. In this study, postcapillary venules with a diameter of 15–20 micrometers were used and the average vessel length was about 80 μ m.

2.6 | Immunohistochemistry (E-selectin and ICAM-1)

E-selectin and intercellular adhesion molecule-1 (ICAM-1) are endothelium-derived adhesion molecules that play potential roles during the course of leukocyte rolling and adhesion onto the endothelium within the postcapillary

FIGURE 2 Representative micrographs showing the sequence of PMN – endothelial interactions on three consecutive images (Panels A–C) recorded by using intravital microscopy (recording rate: 20 frames/s). A segment of the examined postcapillary vein in the urinary bladder is surrounded by lines in Panel A. Movement of rhodamine 6G-labeled PMNs (marked by a–e) is demonstrated frame-by-frame relative to a dashed line. Stationary (adhesive) leukocytes are marked by an ellipse. The bar in Panel C denotes a 100 μ m scale and this applies to all the photomicrographs

venules; hence, we decided to measure their expression in the bladder in response to capsaicin. Forty-five minutes following topical application of capsaicin onto the urinary bladder, the organ was fixed by immersion in a fixative containing 4% paraformaldehyde in a phosphate buffer (0.1 M, pH = 7.4) for 2 h at 4°C. Tissue samples were then transferred into phosphate buffer until further processing. Frozen sections 15 μ m in thickness were cut with a cryostat and prepared for the demonstration of E-selectin and ICAM-1 immunoreactivity using the indirect double labeling immunohistochemical technique. The sections were incubated overnight with mouse anti rat anti-ICAM-1 (clone 1A29; BD Pharmingen, San Jose, CA, 1:1000) and goat anti rat E-selectin (R&D Systems, Minneapolis, MN, 1:1200) primary antibodies dissolved in phosphate buffered saline containing 0.3% Triton X-100. These sections were then incubated with dylight 488 conjugated donkey anti mouse and Cy3-conjugated donkey anti goat secondary antibodies (both from Jackson ImmunoResearch Europe, Newmarket, UK). They were covered with a ProlongGold mounting medium containing DAPI. Immunostained sections were then examined under a Zeiss LSM 700 confocal microscope. One micrometer thin optical sections were collected via Z-stack capturing and the maximal intensity projection function was used to reconstruct an image of the 12 μ m thick block of the tissue. Photomicrographs of control and capsaicin-exposed specimens were taken under identical conditions following a systemic random sampling method. Three bladders per group, three representative sections per bladder, and 5–9 regions per cryosection were selected for a quantitative evaluation. The observer obtained and evaluated photomicrographs of bladder specimens of the control and treated groups in a blinded manner. Relative pixel intensity data of postcapillary venule profiles ($n = 24$ –30 for each experimental group) were collected by using the line profile measurement tool of the

ImagePro Plus 6 image analysis software package (Media Cybernetics, Rockville, MD).

2.7 | Statistical analysis

The minimum number of animals per group was determined based on the “PS: Power and Sample Size Calculator” software package. Data analysis was performed by means of the SigmaStat statistical software package (Jandel Corporation, San Rafael, CA). Changes in microcirculation variables within and between groups were analyzed by two-way repeated measures ANOVA test, followed by the Holm-Sidak test. Immunohistochemistry data were analyzed using the two-way ANOVA test. All data values are expressed as means $\pm$ standard error of the mean (SEM), and P values < 0.05 were considered statistically significant.

3 | RESULTS

The effect of selective sensory chemodenervation and TRPV1 antagonism on the capsaicin-induced microcirculatory changes of the urinary bladder

Topical application of capsaicin resulted in a marked increase in the degree of PMN-endothelial interactions in the postcapillary venules of the urinary bladder. Both primary (rolling, Figure 3A) and secondary interactions (adhesion, Figure 3B) were significantly enhanced within 15 min after topical treatment of capsaicin and remained elevated during the entire observation period of 45 min. Life-long sensory chemodenervation by neonatal capsaicin treatment completely prevented the capsaicin-induced vascular changes and indicated that the vascular effects of capsaicin are not direct, but are mediated through the release of vasoactive

FIGURE 3 Time course of changes in leukocyte rolling (A) and adhesion (B) in the postcapillary venules of the bladder after a 15-min topical exposure to capsaicin or vehicle. In other groups, TRPV1 receptor antagonism (via capsazepine, 200 μ M) and the ablation of TRPV1-positive nerves by neonatal capsaicin treatment were also applied. Values are presented as means $\pm$ SEM. Here, * $P < 0.05$ versus baseline; # $P < 0.05$ versus vehicle

agents from the sensory nerves. Likewise, the co-administration of capsazepine, a competitive TRPV1 antagonist, markedly inhibited the intravascular inflammatory changes elicited by capsaicin. Last, neither capsazepine nor the vehicle for capsaicin produced any changes in PMN-mediated reactions.

The effects CGRP and NK1 receptor antagonists on TRPV1-activation-induced microcirculatory reactions of the urinary bladder

In the urinary bladder, the capsaicin-induced increase in leukocyte rolling was prevented by the topical co-administration of the specific CGRP receptor antagonist CGRP₈₋₃₄ (see Figure 4A), but not by the NK1 receptor antagonist RP67580. However, PMN adhesion was significantly reduced by both the CGRP and the NK1 receptor antagonists (Figure 4B).

The effect of capsaicin on the expression of E-selectin and ICAM-1 in the urinary bladder microvasculature

In control urinary bladders, we noticed a faint-to-moderate staining of small venules with antibodies raised against E-selectin (Figures 5A and 5C) and ICAM-1 (Figures 5B and 5C). Topical application of capsaicin onto the urinary bladder resulted in a massive increase in both E-selectin (Figures 5D and 5F) and ICAM-1 (Figures 5E and 5F) immunoreactivities in small venules. A quantitative evaluation of the staining intensity revealed a significant increase in the relative pixel intensity for both E-selectin (control: 8.11 ± 2.97 ; capsaicin: 35.14 ± 3.45 ; $P < 0.05$) and ICAM-1

(control: 5.45 ± 2.71 ; capsaicin: 33.59 ± 3.24 ; $P < 0.05$) immunostaining.

4 | DISCUSSION

TRPV1 agonism represents one of the possible treatment modalities of overactive bladder syndrome,^{5,6} and the involvement of these afferent nerves in inflammatory processes of the urinary bladder was demonstrated earlier in both animal models⁶ and human diseases such as the painful bladder syndrome and interstitial cystitis.⁷ The cellular inflammatory processes and their dynamics associated with the topical administration of TRPV1 agonists, such as capsaicin and resiniferatoxin, have not yet been investigated. The present study is the first to demonstrate the dynamics of leukocyte-endothelial interactions following TRPV1 receptor activation evoked by capsaicin in the bladder. An early increase in the frequency of endothelial-PMN interactions (both rolling and adhesion) was observed in the bladder postcapillary venules as early as 15 min after the administration of capsaicin and these inflammatory changes persisted throughout the entire experimental period (45 min). The early onset of inflammatory changes following capsaicin administration is in line with previous observations that found a rapid development of NK1 receptor-dependent edema formation and PMN deposition after capsaicin in the skin¹⁰ and in the pancreas,¹⁴ and an even more rapid response occurring within minutes following an intratracheal administration of capsaicin.¹⁹

We have also demonstrated that the microvascular changes were brought about via a specific action of capsaicin on sensory nerves expressing TRPV1 receptors, as capsaicin-induced cellular inflammatory reactions were strongly inhibited by both TRPV1 receptor antagonism and the

FIGURE 4 Time course of changes in leukocyte rolling (A) and adhesion (B) in postcapillary venules of the bladder following a 15-min topical exposure to capsaicin or vehicle. The effects of the selective CGRP antagonist CGRP₈₋₃₇ (10 μ M) and the NK1 receptor antagonists RP67580 (10 μ M) are also shown. Values are presented as means $\pm$ SEM. Here, * $P < 0.05$ versus baseline; # $P < 0.05$ versus vehicle

FIGURE 5 Confocal photomicrographs illustrating the immunohistochemical localization of ICAM-1 (A, D) and E-selectin (B, E) in urinary bladders 45 min after exposure to the vehicle (A, C) and capsaicin (D, F). Note the marked capsaicin-induced increase in immunostaining of both ICAM-1 and E-selectin localized to venules on the lower panels. The merged photomicrographs (C, F) illustrate the close colocalization of ICAM-1 and E-selectin immunoreactivities. The bar in F denotes 50 μ m scale and this applies to all photomicrographs

elimination of TRPV1-expressing nociceptive primary sensory neurons by neonatal capsaicin treatment. These observations suggest a neurogenic origin of the inflammatory process developing in the urinary bladder after the topical administration of capsaicin. The present findings also suggest that the effects of capsaicin are indirect and brought about by the release of pro-inflammatory neuropeptides known to be released from activated TRPV1-expressing sensory nerves.^{1,6} As SP and CGRP are the most abundant vasoactive peptides expressed in intact primary sensory neurons, we focused on the contribution of these neuropeptides to the capsaicin-induced microcirculatory reactions. The findings indicate that both CGRP and SP contribute to the capsaicin-induced endothelial adhesion of PMNs, but rolling is initiated only by CGRP. It is reasonable to assume therefore that TRPV1-induced CGRP release initiates the endothelial-PMN interaction by promoting leukocyte rolling, but adhesion is influenced by both peptides. A similar conclusion was drawn in another study, which showed that SP potentiated neutrophil accumulation in the skin only when it was released at a site where an inflammatory process had already been initiated.²⁰

In the urinary bladder, the capsaicin-induced expression of endothelial adhesion molecules, which play an essential role in

microvascular inflammatory reactions, had not been examined up till now. In our study, the early increase in the expression of E-selectin and ICAM-1 immunoreactivities in the postcapillary venules of the urinary bladder occurred in parallel with the cellular microvascular changes. Previous studies on the human cutaneous vascular bed also demonstrated a very rapid PMN accumulation (in approximately 15 min) after intradermal injection of SP or CGRP²¹ and this is linked to an increased expression of ICAM-1 mRNA (within 60 min after SP exposure).²² Enhanced ICAM-1 and E-selectin protein expressions develop over a longer time-frame (these were typically examined 4 h or more after the topical application of SP in the skin).^{21–23} As for the urinary bladder, the NK1 receptor-mediated expression of ICAM-1 occurred as early as an hour after a local arterial injection of SP.²⁴ Still, little is known regarding the subcellular regulatory mechanism by which the TRPV-related neuropeptide release modulates these changes and the pathway by which capsaicin achieved these effects remained basically unexplored. The remarkably rapid (less than 60 min) increase in the expression of endothelium-derived adhesion molecules demonstrated here probably cannot be explained by the most time-consuming Ca^{2+} -dependent activation of NF- κ B pathways (as shown elsewhere).²³ The involvement of mechanisms related to a capsaicin-induced

intracellular free radical production, however, cannot be ruled out.²⁴ Clearly, the mechanistic background of the particularly rapid adhesion molecule expression in the rat urinary bladder warrants a further in-depth investigation.

The present findings may also be of relevance to neurogenic inflammatory processes that develop in other organs under particular pathological conditions. The dynamics and extent of the inflammatory process may depend not only on the sensory innervation density and the distribution of TRPV1 receptors, but also on other factors such as the density of CGRP and SP receptors and the adhesion molecule expression of the particular tissue/organ.

Last, the potential clinical relevance of the present study should be mentioned, as TRPV1-expressing bladder sensory nerves are important therapeutic targets in the treatment of the overactive bladder syndrome and related disorders of the urinary bladder such as painful bladder syndrome and interstitial cystitis. Quite significantly, in patients with idiopathic detrusor overactivity, the TRPV1 agonist vanilloid compounds like resiniferatoxin and capsaicin are effective only in patients that display an overexpression of the TRPV1 receptor in the urinary bladder.^{7,25} The present findings suggest that, in the urinary bladder, the activation of TRPV1 receptor-expressing nociceptive afferent nerves results in a co-ordinated inflammatory process involving not only arteriolar vasodilatation and plasma extravasation, but also an increase in endothelial-leukocyte interactions that lead to the transmigration of leukocytes. Even though these neurogenic inflammatory changes may be transient due to ensuing rapid defunctionalization/chemodenervation of sensory nerves,⁹ they may interfere with urinary bladder function following intravesical administration of vanilloid compounds for therapeutic purposes. Hence, the inhibition of this inflammatory process by using neuropeptide antagonists (such as CGRP antagonists) which are already approved for other human therapeutic purposes (eg, for migraine approved by the FDA) may be beneficial in the treatment of urinary bladder disorders that utilize vanilloid compounds.

ACKNOWLEDGMENTS

This study was supported by European Structural and Investment Fund grants GINOP-2.3.2-15-2016-00034, EFOP-3.6.2-16-2017-00006, and Hungarian National Research, Development and Innovation Office grant NKFIH K116689. The authors are grateful to Csilla Mester and Nikolett Beretka for their assistance.

CONFLICT OF INTEREST

The authors hereby declare that they had no conflict of interest, financial or otherwise, when they participated in this study.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

How to cite this article: Járomi P, Garab D, Hartmann P, et al. Capsaicin-induced rapid neutrophil leukocyte activation in the rat urinary bladder microcirculatory bed. *Neurourology and Urodynamics*. 2018;37:690–698.
<https://doi.org/10.1002/nau.23376>

PAPER 3

ÖSSZEFOGLALÓ KÖZLEMÉNY

A neurogén hólyag klinikuma és terápiás lehetőségei

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Anyagi támogatás: A kézirat az EFOP-3.6.2-16-2017-00006 támogatásával készült.

Szerzői munkamegosztás: JP: a kézirat elkészítése, BT, BM, PF, SZA: a kézirat szakmai lektorálása

Érdekeltségek: A szerzőknek nincsenek érdekeltségeik.

Összefoglalás

Az alsó húgyutak fő funkciója a vizelet tárolása és ürítése, amely működések zavara az úgynevezett alsó húgyúti tünetegyüttes kialakulásához vezet, ami a kiváltó októl függően vizeletürítési zavarral és vizeletretencióval is járhat. Kezeletlen esetekben a felső húgyutak károsodása következik be a magas hólyagnyomás által kiváltott vesico-ureterális reflux következtében, ami ureter- és veseüregrendszeri tágulat kialakulására, illetve fertőzésekre és kőképződésre hajlamosít. A vizelettárolási/vizeletürítési zavarokat három fő csoportba sorolhatjuk, úgymint stressz (terheléses) inkontinencia, hiperaktív hólyag (nedves/száraz) és neurogén hólyag. A jelen összefoglaló közlemény tárgyát képező neurogén hólyag egy gyűjtőfogalom, mely magába foglal minden releváns neurológiai kórkép talaján kialakult vizelettárolási és vizeletürítési zavart. Mivel a húgyhólyag mellett a záróizomzat és a hátsó húgyecső is érintett, ezt a kórképet napjainkban „a neurogén alsó húgyúti diszfunkció” elnevezéssel is szokás illetni. A kórállapotot a neurológiai diszfunkciók széles spektruma okozhatja, kezdve a helyi funkcionális zavartól, a helyi idegi sérülésen át a felső és alsó motorneuron sérülésig, vagy a centrális degeneratív folyamatokig. Az eltérő etiológia ellenére a klinikai tünetek rendszerint két alapvető klinikai típusban manifestálódhatnak: túlműködő (fokozott detrusor-kontraktilitást okozó automata) hólyag vagy alulműködő hólyag formájában. Tekintettel a neurogén alsó húgyúti diszfunkció következtében létrejövő felső húgyúti komplikációkra, a közlemény egyik célja a betegség diagnózisát segítő algoritmus bemutatása a legújabb nemzetközi szakirodalmi ismeretek alapján. A neurogén hólyag kezelése jobbra nem terjedhet ki a kiváltó ok kezelésére, ezért a jelen összefoglaló másik célja azon gyógyszeres és invazív terápiás beavatkozások összefoglalása, melyek a felső húgyutak védelmét szolgálják az alacsony hólyagnyomás fenntartása révén.

Kulcsszavak: alsó húgyúti tünetegyüttes, késztetéses inkontinencia, túlműködő hólyag, alulműködő hólyag

Clinical aspects and therapeutic possibilities of neurogenic bladder

Summary

Storage and urination are the main functions of the lower urinary tract and its lesions lead to the so-called lower urinary tract syndrome causing either urinary incontinence or retention. In untreated cases, the upper urinary tract becomes injured via a vesico-ureteral reflux resulting from increased bladder pressure and resultant dilations of the ureter and the renal pelvis which predispose to infection and stone formation. Lower urinary tract storage/urination disorders can be classified as stress incontinence, hyperactive bladder (wet/dry) and neurogenic bladder. Neurogenic bladder which is the subject of this review, is a collective term that encompasses all urinary storage and emptying disorders which develop on the basis of neurological diseases. Being not only the bladder, but also the sphincter and posterior urethra (generally termed as the "bladder outlet") affected, nowadays this condition is referred to as "neurogenic lower urinary tract dysfunction". A wide range of neurological dysfunctions could contribute to the development of this condition, ranging from local dysfunction (autonomic dysreflexia) or local nerve injury to upper/lower motoneuron injury or central degenerative processes. Regardless the diverse etiology, the clinical symptoms eventually manifest in two major forms i.e. overacting (automatic bladder with increased detrusor contractility) and underactive bladder. Considering the severity of complication occurring in the upper urinary tract in response to the pathophysiological changes in the lower urinary tract, one of the aims of this paper was to present an algorithm aiming to build up a state of the art diagnosis of the disease based on current international literature data. Since treatment of the neurogenic bladder usually can not target elimination of the underlying cause, the other goal of the present paper is to summarize the pharmacological treatment regimen and invasive therapeutic interventions that protect the upper urinary tract by maintaining low pressure values in the bladder.

Keywords: lower urinary tract syndrome, urge incontinence, overactive bladder, underactive bladder

Rövidítések

CT = computertomographia;

DSD = detrusor sphincter disszinergia;

EMG = electromyographia;

EQ-5D = EuroQol 5 Dimensions Questionnaire;

KHQ = King's Health Questionnaire;

LUTS = Lower Urinary Tract Symptoms;

NLUTD = Neurogenic Lower Urinary Tract Dysfunction;

PPBC = Patient Perception of Bladder Condition;

PPIUS = Patient Perception of Intensity of Urgency Scale;

TRPV = transient receptor potential vanilloid

A neurogén hólyag klinikai háttere és tünetei

Normális alsó húgyúti működés esetén a vesék által kiválasztott vizet a húgyhólyag tárolja, majd megfelelő időközönként üríti. A tárolási fázisban a húgyhólyag relaxált állapotban van, majd a volumen növekedésével a központi idegrendszerben kialakuló hólyag-telődés érzetre adott válaszként akaratlagosan történik a vizeletürítés. Ez a húgyhólyag izomzatának összehúzódásával a záróizom egyidejű ellazulása révén valósul meg. A fenti működések zavara vagy sérülése azonban vizelettárolási és ürítési zavarok formájában manifesztálódik, amit az urológiai gyakorlatban ún. alsó húgyúti tünetegyüttesként (angolul Lower Urinary Tract Symptoms=LUTS) definiálnak. A vizelettárolási és ürítési zavarokat három fő csoportba sorolhatjuk, úgymint stressz inkontinencia, hiperaktív hólyag és neurogén hólyag. A jelen összefoglaló közlemény tárgyát képező neurogén hólyag egy gyűjtőfogalom, mely tulajdonképpen magába foglal minden releváns neurológiai kórkép talaján (lásd később) kialakuló vizelettárolási és vizeletürítési zavart, így a fent említett önálló kórképek (stressz inkontinencia és hiperaktív hólyag) tüneteit is hordozhatja. Ezekben a folyamatokban a húgyhólyag mellett a záróizomzat és a hátsó húgycső is érintett (mely utóbbiak megnevezése az angol terminológia alapján összefoglalóan „bladder outlet”), ezért ezt a kórképet napjainkban „a neurogén alsó húgyúti diszfunkció” (angolul „Neurogenic Lower Urinary Tract Dysfunction=NLUTD) elnevezéssel illetjük. A jelen összefoglalóban az idegen nyelvű rövidítés használatának elkerülése céljából és meglehetősen hosszú „a neurogén alsó húgyúti diszfunkció” elnevezés helyett a klasszikus „neurogén hólyag” kifejezést használjuk a tágabb fenti fogalmi értelmezés mellett.

A kórkép magában foglalja a neurológiai eredetű diszfunkciók széles spektrumát, betegek millióit érintve világszerte. A lehetséges etiológia sokszínűségét jelzi, hogy kórkép kialakulhat a helyi funkcionális zavarok (pl. autonóm dysreflexia, detrusor sphincter disszinergia (DSD), helyi (például kismencedei műtetet követően perifériás) idegsérülések, felső és alsó motorneuron sérülések (harántlaesiók) vagy degeneratív idegrendszeri folyamatok következtében egyaránt. A leggyakoribb idegrendszeri eltéréseket okozó betegségek a spina bifida (több, mint 95%-os valószínűséggel) [1], a gerincvelő sérülések (90%-ában) [2], a sclerosis multiplex (az esetek 50-80%-ában) [3,4], de stroke, Parkinson kór továbbá akár cukorbetegség, infekciók vagy mérgezések is állhatnak a háttérben [5]. A fentiek a húgyhólyag, a záróizom, az afferens és efferens idegek, a gerincvelő és a központi idegrendszer összehangolt működésének zavarát eredményezik.

A neurogén hólyag a kiváltó októl függően vizelettárolási és vizeletürítési zavarral és következményes vizeletretencióval járhat. Kezeletlen esetekben a felső húgyutak károsodása jön létre a magas hólyagnyomás miatt létrejövő vesico-ureterális reflux következtében, ami ureter és veseüregrendszeri tárgulat kialakulására, illetve fertőzésekre és kőképződésre hajlamosít.

A neurogén alsó húgyúti diszfunkció az idegrendszeri érintettség lokalizációja szerinti tünetegyüttest okozhat. A leggyakoribb tünet a késztetési inkontinencia, amihez a kórkép lokalizációjától függően vizeletürítési zavar és/vagy vizeletretenció társulhat. A tünetek hátterében a hólyag csökkent tágulékonyága (compliance-e), túlműködő (overactive) vagy alulműködő hólyag, vizeletrekedés vagy a detrusor és a záróizom disszinerg működése állhat. A fentiek a felső húgyutak károsodásához vezethetnek a kialakuló hólyagnyomás-emelkedés, vesico-ureterális reflux és a következményes ureter és veseüregrendszeri tágulat miatt, melyek fertőzésekre és kőképződésre hajlamosítanak [6]. A beteg életminőségét jelentősen befolyásoló betegség kihat a mentális egészségre, alvásra, munkahelyi teljesítményre és növeli a húgyúti fertőzések rizikóját. A diagnózis gyors és pontos felállítása fontos a vesekárosodás és az infekciók megelőzése szempontjából egyaránt [7]. A terápia legfontosabb célja a felső húgyutak védelme (így a vesekárosodás és uroszepszis megelőzése), a hólyagnyomás és a visszamaradó vizeletmennyiség csökkentése révén. Ezek teljesülése és a kontinencia lehetséges visszaállítása révén pedig a betegek életminősége is javul [8].

A jelen összefoglaló közleményben a rendelkezésre álló szakirodalmi források adatait ötvözve felvázolunk egy differenciáldiagnosztikai algoritmust, ami az alap-, és a szakellátás területén dolgozó kollégák munkáját egyaránt segítheti. Szintén felvázolásra kerülnek a kórkép jelenlegi gyógyszeres és invazív terápiás lehetőségei.

A neurogén hólyag diagnosztikai lehetőségei

A neurogén hólyag esetén gyakran észlelünk késztetési inkontinenciát, amihez vizeletürítési zavar és vizeletretenció is társulhat. A túlműködő hólyagot idiopathiás, neurogén, illetve miogén csoportba sorolhatjuk a kiváltó októl függően. A neurogén csoport esetén a tünetek jellegét a laesio elhelyezkedése határozza meg. A felső motoneuron (suprapontin és infrapontin-suprasacralis) laesiók detrusor túlműködést okoznak, míg az alsó motoneuron (sacralis-infrasacralis és cauda equina) szintjében bekövetkező és a perifériás idegeket érintő sérülés petyhüdt hólyagot eredményez alulműködő detrusor izommal és csökkent teltségérzékeléssel jár. A pontin és a sacralis vizelési központ közötti laesiók DSD-t okoznak. Ennek létrejöttében a reflexív szenzoros ágában a capsaicin-szenzitív C-afferens rostokon keresztül megvalósuló automácia is szerepet játszhat. Mindkettő idegrendszeri folyamat következménye hasonló lesz, hiszen a magas hólyagnyomás vesico-ureterális reflux, hydronephrosis és pyelonephritis formájában manifesztálódik [6,9].

Kivizsgálás

Első lépésként fontos a pontos anamnézis, ami a tünetek kialakulásának időpontjára, az alsó húgyúti tünetekre (a vizelet gyakoriságára, a „vizelet sugara”, vizelettartási panaszok) és az alkalmazott gyógyszerek feltérképezésére irányul (**1. Táblázat**)[10]. A tünetek súlyosságának objektív megítélésében validált kérdőívek és vizelési napló segíthetnek [11].

A fejlettebb országokban széleskörűen elterjedt validált kérdőívek Magyarországon limitáltan érhetőek el. Ezek a betegek kivizsgálásában és utánkövetésében is használhatóak a beteg általános egészségi állapotának megítélése ([King's Health Questionnaire \(KHQ\)](#), [EQ-5D](#)), illetve betegspecifikus (életminőség) felmérése révén ([Sf-qualiveen](#), [Patient Perception of Bladder Condition \(PPBC\)](#), [Patient Perception of Intensity of Urgency Scale \(PPIUS\)](#), [Work Productivity and Activity Impairment](#)) [12]. A vizelési napló, melyet a beteg három-hét napon keresztül vezet, segít felmérni a vizelet gyakorisága és az ürített mennyiség közötti összefüggést.

Fizikális vizsgálat után laborvizsgálatok (vizelet üledék, vesefunkció), illetve képalkotó vizsgálatok (ultrahang, CT, vesescintigraphia) végzése szükséges [6]. A neurológiai vizsgálatok során reflexvizsgálatok segítik a laesio helyének meghatározását [13]. Urodinámiai vizsgálatokat is rutinszerűen végzünk a hólyag és a záróizom funkciók vizsgálata céljából, melyek során nemcsak a vizelettárolási, hanem a vizeletürítési funkciókról is információt kapunk. Az urodinámiai vizsgálatok a neurogén hólyaggal rendelkező betegek esetében kötelezően elvégzendő vizsgálatok, melyek az utánkövetésben és a kezelés eredményességének felmérésében is nélkülözhetetlenek. Míg az uroflowmetria és a cystometria rutinszerűen elérhető Magyarországon, addig az elektromiográfia csak korlátozottan, valamint a videó-urodinámia (mely az Európai Urológus Társaság által jelenleg elsődlegesen javasolt vizsgálat neurogén hólyagnál)[10] tudomásunk szerint (a Pécsi Tudományegyetem Urológiai Klinikáját leszámítva) jelenleg nem érhető el hazánkban.

A non-invazív vizsgálatok közül az uroflowmetriával deríthetjük fel a vizelet dinamikáját a vizeletürítési sebesség és az ürített vizeletmennyiség közötti kapcsolatra alapozva [8](**1. Ábra**). Ez a vizsgálat beteg utánkövetésében is nagy segítséget nyújthat.

Cystometria során nyomás-volumen görbék analízise révén, invazív módon mérjük fel a húgyúti traktus funkcionális állapotát (az esetleges húgyúti fertőzések előzetes szigorú eliminálását követően) [6,14]. A hólyagon belüli nyomásviszonyok analízise révén a detrusor izom és sphincter működését vizsgáljuk a húgyhólyagba és (a hasúri nyomás mérése céljából) végbélbe vagy hüvelybe helyezett, nyomásérzékelővel ellátott katéterek és a gát izmaira rögzített elektródák segítségével. A mesterségesen létrehozott töltési fázis során katéteren keresztül (8-20 ml/perc áramlási sebességgel)

testmeleg fizioiógias sóoldattal töltjük a hólyagot, a kezdeti telődés-érzet, az erős és sürgető vizelesi inger fázisainak detektálása mellett (2. Ábra). A mérési adatokból a hólyag compliance (ami a térfogat- és nyomásváltozás hányadosából számítható) és a töltési fázis végén elérhető maximális térfogat, azaz a cystometriás kapacitás határozható meg. A kisebb kapacitás (< 200ml) és magasabb detrusor (> 75 H₂Ocm) nyomás (ún. „high pressure bladder”) a felső húgyutak károsodásának fontos rizikófaktorai [10]. A detrusor izom akaratlan összehúzódásait is ki tudjuk mutatni, hiszen normális körülmények között a töltöttség különböző szintjei ellenére sem változhat a hólyagon belüli nyomás (még olyan esetekben sem, mint hidegvízzel kivitelezett vagy gyors hólyagtöltés, illetve a helyzetváltoztatás). A vizsgálatnak azonban súlyos, akár életet veszélyeztető szövődményei lehetnek (jellemzően a háti 6-os csigolya feletti laesio esetén). Az ún. autonóm diszreflexia esetén ugyanis hirtelen jelentkező fejfájás, arcpír, verejtékezés, a szisztolés vérnyomás 20 Hgmm-nél nagyobb mértékű emelkedése, sőt akár hypertenzív krízis is kialakulhat. Ezért ezen betegek szoros monitorizálása szükséges a vizsgálat során [10].

Electromyographiás (EMG) vizsgálatok során a külső húgycső sphincter, a peri-urethralis izmok és a medencefenék, ill. anus akaratlagos izmait vizsgáljuk annak felmérése céljából, hogy a beteg miképp tudja kontrollálni a medencefenéki izmok működését, melyek mind anatómiailag, mind funkcionálisan összefüggnek, így igazolhatjuk a laesio okozta eltérés jelenlétét, a centrális vagy perifériás motoneuron sérülést és a laesio kiterjedését [15,16].

Videó-urodinámiás vizsgálatok során a nyomás-áramlás vizsgálatokat egészítjük ki képalkotó vizsgálatokkal, melyek az alsó húgyutak patológiás eltérésit és a felső húgyutakra kifejtett hatását, (pl.: reflux) képesek vizualizálni [17-19]. A video-cystographia a vizeletürítést folyamatában rögzíti, így különösen a hólyagnyak disszinergia, a belső sphincter illetve a hólyagnyak szűkülete detektálására alkalmas.

A fentiek alapján az alsó húgyúti működések objektív vizsgálatában az invazív urodinamikai vizsgálatok segítenek; a tárolási funkciókról a töltéses cystometria, az ürítési funkciókról pedig a nyomás-áramlás vizsgálat tájékoztat. Mind a szabad uroflowmetria, mind a nyomás-áramlás vizsgálat során végzett uroflowmetria kiegészíthető EMG-vel, a DSD finomabb értékelése céljából [20].

A neurológiai vizsgálatok célja az idegrendszeri laesio lokalizációjának meghatározása. A leggyakrabban vizsgált reflexek közül a bulbocavernosus (L5-S5), a cremaster (L1-2) és az anocutan reflex (S3-5) jelenléte kizárja az alsó motoneuron sérülést. Ezek mellett a végbél-sphincter tónusának vizsgálata során a csökkent tónus alsó motoneuron laesióra, míg a fokozott tónus felső motoneuron laesióra utal.

Az előzőekben részletezett diagnosztikus séma útmutatást adhat/algorithmust szolgáltat a neurogén hólyag diagnózisának és differenciáldiagnózisának felállításához, azonban figyelembe kell venni bizonyos specifikumokat. Ami a tüneteket és anamnesztikus adatokat illeti, számos betegség okozhat akár a felső-, akár az alsó motoneuron sérüléshez hasonló tüneteket a fenti neurológiai tünetek hiányában is, így a diagnosztikának vannak limitációi [7]. Ugyanígy számos betegség okoz hasonló urodinamias eltéréseket neurogén komponens nélkül is, nem is beszélve a pyuria, vizelet retenció, vizelet reflux vagy pyelectasia számos potenciális okától. Neurogén hólyag esetén a tünetek enyhítése csak bizonyos kórképek esetén érhető el az alapbetegség kezelésével (például myelitis, Parkinson, sclerosis multiplex), általában oki kezelés nem valósítható meg, ami felhívja a figyelmet arra, hogy az urológiai gyakorlatban az azonos tünetcsoporttal járó, de különböző etiológiájú betegségek kezelése főként tünetek befolyásolása irányában történik, így a kiváltó októl független lehet. Így a továbbiakban olyan terápiás lehetőségeket mutatunk be, mely a kiváltó októl függetlenül a neurogén hólyag szindróma tüneteinek enyhítésére szolgálhatnak.

A neurogén hólyag gyógyszeres és invazív terápiás lehetőségei

1. Gyógyszeres kezelés

A neurogén hólyag esetén oki kezelés nem végezhető, a tüneti kezelésben használt gyógyszercsoportok más nem neurogén eredetű kórképek kezelésére lettek törzskönyvezve [8]. A második táblázatban a legfontosabb hatástani csoportokat mutatjuk be (**2. Táblázat**).

Antimuscarinerg szerek

A leghosszabb ideje használt és az Európai Urológus Társaság által is javasolt gyógyszercsoport a felnőttek és gyermekek neurogén hólyagbetegségeiben egyaránt. Ezek az acetilkolin kompetitív antagonistái a muscarinerg receptorokon [8]. Az 2-es táblázatban felsorolt szerek a hármas típusú muscarinerg receptorhoz kötődve gátolják a detrusor izom akaratlan összehúzódását (**2. Táblázat**). Ezen receptorok a legújabb kutatások szerint az urotheliumban is jelen vannak, így a szenzoros működést is befolyásolják. A dózis növelésével növelhető a terápiás hatékonyság is [21]. Oxybutinin leggyakoribb mellékhatása a szájszárazság, mely a többi hatóanyagnál a nyálmirigy muscarinerg receptoraihoz való csökkent affinitásuk miatt kevésbé jelentkezik. Általánosságban elmondható, hogy a hosszabb felszívódású készítményeknél kevesebb mellékhatás lép fel. Az antimuscarinerg szerek a vér-agy gáton átjutva a kognitív funkciókat is gátolhatják, amit a betegek egyéb gyógyszereinek (calcium-csatorna blokkolók, opioidok, izomrelaxánsok, antidepresszánsok, dopamin agonisták és

antihisztaminok) következtében kialakuló antikolinerg terhelése miatt tovább fokozhat. Ezek szedése zárt zugú glaucoma és gastroparesis esetén kontraindikált, myasthenia gravisban pedig alkalmazásuk pedig csak nagy körültekintéssel végezhető.

Alfa-blokkolók

Az alfa adrenerg receptor blokkolók a benignus prosztata hyperplasia kezelésében terjedtek el széleskörűen az urológiai gyakorlatban, de neurogén hólyaggal rendelkező betegek esetében, akik képesek vizeletürítésre, szintén javuló uroflowmetriás és maximális detrusor nyomás értéket mutatnak ezen gyógyszerek hatására, a reziduális vizeletmennyiségre való hatás nélkül (**2. Táblázat**). Gerincvelő sérülés során kialakult autonóm diszreflexia esetén is javulás érhető el ezzel a kezeléssel. A mellékhatások közül kiemelendő az orrdugulás, az ejakulációs zavar, a szédülés és a hipotenzió.

Triciklusos antidepresszánsok: Imipramin

Manapság ez egy kevésbé gyakran használt triciklusos antidepresszáns, mely muscarin receptor antagonist és béta receptor agonista hatásánál fogva közvetlenül gátolja a hólyag simaizomzatának összehúzódását (**2. Táblázat**). Korábban főként enuresis nocturna és neurogén detrusor túlműködés kezelésben mutatott jó eredményeket [22].

Kolinészteráz enzim bénítók: Piridostigmin

Ez elsősorban izomgyengeség (pl. myasthenia gravis és különböző eredetű bélrenyheség) kezelése vonatkozásában elterjedt készítmény, ami neuro-urológiai betegségekben is hatásos lehet a detrusor izom összehúzódásának segítése révén (mely hatás az acetilkolin lebontás gátlása révén valósul meg).

Adrenoceptor agonista: Mirabegron

Jelenleg ez az egyetlen β_3 adrenoceptor agonista, mely forgalomban van a detrusor túlműködés kezelése céljából (**2. Táblázat**). Mellékhatásprofilja kedvezőbb, mint az antimuscarinerg szereknek. Mellékhatásként jelentkezhet enyhe vérnyomás- és szívfrekvencia emelkedés, így nem kezelt hipertóniás betegeknél nem alkalmazható [23].

Hormonális kezelések: Desmopressin

A gerincvelő magasabb szintjén sérült betegeknél, enuresis nocturnában szenvedő gyerekeknél és a vazopresszin termelődésének hiánybetegségeiben alkalmazható készítmény [24] (**2. Táblázat**). Autonóm diszreflexia esetén a nocturális polyuria csökkentése miatt választandó ez a kezelés, míg

sclerosis multiplexben széleskörűen tanulmányozott hatóanyag vizelettermelődést csökkentő hatása miatt, mely szignifikáns szérumban nátrium szint csökkenés nélkül valósul meg [25].

A vizelési reflex szenzoros és motoros innervációjára ható kezelések

Azon betegeknél, ahol a szájon alkalmazható gyógyszeres kezeléssel nem érünk el megfelelő hatást, a reflexív szenzoros és motoros működéseinek lokális befolyásolásával is érhetünk el átmeneti eredményt botulinum toxin intravesicalis instillációja révén (**2. Táblázat**). A klinikai gyakorlatban erre a célra korábban capsaicint és a resiniferatoxint is használtak. A szenzoros ág esetében az ún. transient receptor potential vanilloid (TRPV) csatornák jelentősége is bizonyított a neurogén detrusor túlműködés eseteiben. Ekkor főként a TRPV1 és TRPV4 receptor altípusok fokozott expressziója mutatható ki, aminek szerepe lehet a húgyhólyagban bekövetkező megnyúlás fokozott szenzoros érzékelésében [26]. A TRPV receptorok capsaicinnal történő gátlása révén sikerült a kóros vizelési reflexműködéseket mérsékelni [27,28] részben a C-afferensekhez kötődő idegvégződések neuropeptid szintjének csökkenését [29], részben pedig a TRPV1 receptorok deszenzitizációját illetve roncsolását feltételezve a kedvező hatások hátterében [30,31], mely hatások resiniferatoxinnal is kiválthatók [32-34]. Jelenlegi gyakorlatban a motoros működések botulinum toxin intravesicalis alkalmazása révén befolyásolhatók, melynek hatása a preszinaptikus acetilkolin felszabadulás gátlásán keresztül valósul meg, a cystometriás kapacitás növelése és az inkontinens epizódok számának, illetve a detrusor nyomás csökkentése révén [35-36]. Mind a detrusor izom, mind a sphincter ellazítása elérhető botulinum toxinnal, így mind az alsó, mind a felső motoneuron laesio esetén alkalmazható lehet. Mellékhatásaik közül a vizeletretenció és a fertőzések emelendők ki, illetve sphinctericus alkalmazás esetén stressz inkontinenciával lehet számolni. Ezek a kezelések átlagosan 6-9 hónapig mérséklék/szüntetik meg a tüneteket.

2. Invazív kezelési módok

Jelenleg nem állnak rendelkezésre átfogó tanulmányok és erős evidenciák, melyek a tünetek mérséklésének hatékonyságát meggyőzően támasztanak alá a nervus pudendus elektrostimulációja és a következményes vizelési reflex / detrusor izomzat gátlás vonatkozásában [37](**3. Táblázat**). Ez történhet a tibialis idegnek bőrön keresztüli ingerlésével, kiegészítve a medencefenék izmainak stimulációjával. A hólyagon belüli (intravesicalis) stimuláció a hólyagkapacitás növelése és a hólyagürítés javulása révén lehet kedvező hatású [38].

A reziduális vizelet csökkentésének eszköze az intermittáló steril vagy « tiszta katéterezés » (vagy önkatéterezés). A felső húgyutak károsodásának megelőzése alapvető fontosságú a kezelési módok

megválasztásában. Emiatt szükségessé válhat a hólyagnyak gyógyszeres (botulinum toxin) vagy sebészi (urethrotomia, sphincterotomia) denervációja, illetve ballonos dilatációja.

A stressz inkontinens panaszok javítása érdekében húgycső sling/szalag (fix, ill. állítható) alkalmazható, míg a vizelettartás javítása miatt műsphincter vagy autológ szövet (angolul fascial sling) ültethető be, amennyiben nincs emelkedett nyomás a hólyagban és nem tapasztalunk refluxot.

A detrusor izom kontrakcióját a Brindley-féle sacralis ideg stimulációjával érhetjük el szelektált beteganyag esetében, míg detrusor túlműködés esetén sacralis rhisotomiát végezhetünk.

Azon stabil neurológiai betegséggel rendelkező, nem komplett harántlaesiós betegeknél, akiknél maradék detrusos aktivitás kimutatható, a Magyarországon is elérhető sacralis neuromoduláció mind a tárolási tünetek javításában, mind vizeletretentio jelentkezése esetén eredményes eljárás lehet.

A hólyag-augmentáció történhet a hólyag detrusor izomzatának bemetszésével vagy béllal történő megnagyobbításával. A beavatkozás hosszú utánkövetése a metabolikus eltérések miatt fontos, de szövömenyként jelentkezhet köképződés és perforáció is. A felső húgyutak védelmében szükségessé válhat a vizelet elterelése, ami megvalósulhat kontinens sztóma segítségével, mely katéterezést tesz szükségessé, illetve inkontinens sztóma kialakításával [37,39].

Összegzés

A fent vázolt diagnosztikai algoritmus segíthet a laesio lokalizációjának meghatározásában (neurológiai vizsgálat) és annak tisztázásában, hogy „túlműködő vagy petyhüdt típusú” neurogén hólyaggal állunk-e szemben (urodinámiás kivizsgálás). Ez elsősorban a neuro-urológia iránt érdeklődő kollégák számára lehet hasznos a neurológus, urológus és rehabilitációs szakember képzés során. A fő cél a felső húgyutak károsodásának megakadályozása a beteg szubjektív tüneteinek mérséklése mellett (az urgens vizeletürítés csökkentése és a kontinencia javítása révén). A diagnózis tehát részben a laesio helyének pontos meghatározásából, részben pedig a hólyagban fennálló nyomásviszonyok vizsgálata révén, a kórállapot súlyosságának megállapításából áll. Az általunk javasolt sorrendre alapozva lehetőség van az urológia kivizsgálás optimalizálására és hatékonyságának fokozására, a diagnózis szempontjából nélkülözhetetlen vizsgálatokra fókuszálva. A vizsgálati eredmények a minimális vizeletretenciótól a legsúlyosabb stádiumnak számító hydronephrosisig terjedhetnek. Az anamnézis, a rutin vizeletvizsgálat, sőt a rutin ultrahang vizsgálat sem vezet el a két típus elkülönítéséhez (hiszen ez utóbbi is csak a vizeletretenció tényét igazolja mindkét típus esetén), hanem invazív urodinámiás vizsgálatokra van szükség, melyek a vizeletürítés dinamikus, funkcionális állapotváltozásait és ezek súlyosságát hivatottak feltérképezni. Ezek a vizsgálati eredmények a megfelelő és optimális (sokszor individualizált) terápia megválasztását szolgálják, a lehetséges szövődmények minimalizálása mellett.

A megfelelő hatás elérése mellett a mellékhatások minimalizálása és relatíve jó tolerálhatósága a fő célkitűzés, mely jelen tudásunk szerint a legnagyobb arányban az antimuscarinerg szerek esetében figyelhető meg [21]. Az alfa adrenerg blokkoló gyógyszereket ebben a betegpopulációban vizeletürítési nehézség, sphincter túlműködés, illetve detrusor hypofunkció esetén segíthetik a vizeletürítést [20]. Az antimuscarinerg szerek bár a tárolási tüneteket javíthatják, ugyanakkor retencióhoz vezethetnek, amit az alfa adrenerg blokkoló gyógyszerekkel előzhetünk meg, kombinált kezelés formájában [20]. A hólyag compliance fokozható továbbá imipramine alkalmazásával, a fenti hatóanyagokkal való kombinációban. A megfelelő gyógyszeres kezelés megválasztásával a betegek tüneteinek 63%-kal javultak a placebo mellett megfigyelt 22%-os eredményhez képest [21]. A tünetek enyhítésére (nemzetközi szakirodalmi adatok szerint) cannabinoidok és szelektív szerotonin-noradrenalin visszavétel gátlók is szóba jöhetnek. A vizeleti reflex szenzoros ágának befolyásolását ma már nem használják (capsaicin és resiniferatoxin révén) kevésbé kontrollálható hatásuk miatt [31-35]. A detrusor izom denerváció gyógyszeres terápiára rezisztens esetekben jön szóba botulinum toxin lokális alkalmazása mellett [36]. A konzervatív kezelés hatástalansága vagy komoly mellékhatásai miatt jönnek szóba az invazív kezelési módok, ezeket azonban a műtéti szövődmény-ráta ismeretében kell mérlegelni. Az invazív kezelési módok közül sok esetben továbbra is katéterezés/önkatéterezés szolgálhatja a felső húgyutak védelmét.

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Ábramagyarázatok

1. ábra. Egy egészséges személy uroflowmetriás regisztrátuma

2. ábra. A felső (A) és alsó (B) motoneuron laesio tipikus cystometriás képe. A: akaratlan detrusor összehúzódások intravesicalis nyomásemelkedéssel (detrusor disszinergiában szenvedő betegen. B: nincs nyomásemelkedés a töltés hatására, összehúzódások hiánya (emiat spontán uroflow regisztrátum nem készíthető), C: haspréssel történő vizelés uroflowmetriás képe (forrás: saját regisztrátum).

1. Táblázat. Javasolt vizsgálati algoritmus neurogén hólyag szindróma esetén a javasolt kivizsgálási módszerek sorrendjében (a nemzetközi szakirodalom alapján összegezve, illetve saját klinikai tapasztalat alapján)[10]

	I. típusú beteg: hiperaktív hólyaggal (felső motoneuron sérült; lumbo-sacralis régió feletti sérült pl. stroke és trauma)	II. típusú beteg: petyhüdt hólyaggal (alsó motoneuron sérült; lumbo-sacralis vizelési központ alatti sérült pl. trauma)
Tünetek háttérében álló pathomechanizmus	akaratlan detrusor összehúzódás, DSD, nyomásemelkedés	alulműködő detrusor, retenció, reflux
Differenciáldiagnosztika	<ul style="list-style-type: none"> - (idiopátiás) hiperaktív hólyag - interstitialis cystitis - cystitis (és a húgyúti rendszer fertőzései) - cyclophosphamid-indukálta cystitis - hólyag tumor 	<ul style="list-style-type: none"> - prostata hyperplasia - húgycső szűkület - székrekedés - kismencedei tumor
1. Vizsgálat: anamnézis, klinikai tünetek	önműködő hólyag („reflex hólyag”) +/- szakaszos vizeletürítés -DSD esetén	+/- sürgető vizelési inger +/- gyakori vizelés +/- akaratlan vizeletvesztés
2. Rutin urológiai fizikális vizsgálat	+/- retenció +/- vesetáji érzékenység	+/- retenció +/- vesetáji érzékenység
3. Vizeletvizsgálat	+/- pyuria +/- bakteriuria	pyuria (+/-microszkópos hematuria (+/- bacteruria)
4. Ultrahang (hólyag és vese)	+/- retenció +/- pyelectasia	retenció +/- pyelectasia
5. Urodinámiás vizsgálat (1-2. ábra)	hólyag telődés közben akaratlan detrusor összehúzódások, disszinerg sphincter-működés	hólyag telődés közben nyomásemelkedés nagyobb volumeneknél következik be, disztendált, alacsony nyomású, nem kontraktilis hólyag
6. Electromyographia	csökkent/nincs akaratlagosan kiváltható elektromos aktivitás, reflex aktivitás változatlan	az akaratlagos és reflex aktivitás csökkenése vagy teljes hiánya, denervációs potenciál, fibrilláció, fascikuláció
7. (Video-) Urodinámiás vizsgálatok (uroflowmetria) (1-2. ábra)	vizeletürítés: kisebb volumen ürül, előfordulhat szakaszos vizeletürítés	vizeletürítés: csökkent áramlási sebesség, elnyújtott vizelés, haspréssel, műfogással
8. Neurológiai vizsgálat	bulbocavernosus reflex ép,	bulbocavernosus reflex

	végbélsphincter tónus fokozott	károsodott, végbélsphincter tónus csökkent
9. Kiegészítő vizsgálatok (differenciáldiagnosztikai célból pl. tumor kizárására)	cystoscopy, CT, (cysto-urethrographia)	vesescintigraphia, mictios cysto-urethrographia, retrograd cystographia (reflux kimutatása)

2. Táblázat. Gyógyszeres terápia a neurogén hólyag kétfajta alapesetében a klinikai alkalmazási gyakoriság sorrendjében [20]

I. típusú beteg: hyperaktív hólyaggal	II. típusú beteg: petyhüdt hólyaggal
Cél: a hólyag detrusor izom relaxációja az acetilkolin receptorok lokális gátlásán keresztül Antimuscarineg szerek (napi dózis): <ul style="list-style-type: none"> - Oxybutinin (5-30 mg) - Solifenacin (5-10 mg) - Trospium (20-60 mg) - Darifenacin (7,5-15 mg) - Tolterodine (2-8 mg) - Fesoterodin (4-8 mg) 	Cél: a hólyagalap és a hólyagnyak/húgycső simaizom relaxációja az alfa-2 receptorok lokális gátlásán keresztül Alfa-blokkolók (napi dózis): <ul style="list-style-type: none"> - Tamsulosin (0,4-0,8 mg) - Alfuzosin (5-10 mg) - Doxazosin (2-8 mg) - Terazosin (2-10 mg) - Silosodin (8 mg)
Cél: a hólyag detrusor izom relaxációja az acetilkolin receptorok centrális gátlásán keresztül Triciklusos antidepresszánsok (napi dózis): <ul style="list-style-type: none"> - Imipramin (10-45 mg) 	
Cél: a hólyag detrusor izom relaxációja az beta-3 adrenoreceptorok serkentésén keresztül Beta 3-receptor agonista (napi dózis): <ul style="list-style-type: none"> - Mirabegron (25-50 mg) 	
Cél : a vizeletelválasztás csökkentése hormonális úton a vazopresszin útvonal befolyásolása révén (napi dózis): <ul style="list-style-type: none"> - Desmopressin (25-50 mg) 	
Cél: a hólyag detrusor izom motoros bénítása az acetilkolin felszabadulás gátlásán keresztül <ul style="list-style-type: none"> - Botulinum toxin (100-300 U a hólyagfalba injektálva) 	Cél: a sphincter motoros bénítása <ul style="list-style-type: none"> - Botulinum toxin (100-300 U a sphincterbe injektálva)

3. Táblázat. Invazív kezelési módok a neurogén hólyag szindróma kétfajta alapesetében a klinikai alkalmazás sorrendjében (a nemzetközi szakirodalom alapján összegezve [37], illetve saját klinikai tapasztalat alapján)

I. típusú beteg: hiperaktív hólyaggal	II. típusú beteg: petyhüdt hólyaggal
Percutan idegstimulációs kezelések	Katéterezés és önkatéterezés
Sacralis neuromoduláció	Intravesicalis elektrostimuláció
Urethrotomia/sphincterotomia	Sacralis neuromoduláció
Hólyag augmentáció	Urethrotomia/sphincterotomia
Vizeletelterelés (sztóma)	Műsphincter, fascial sling
Detrusor izom (későbbiekben esetleg sphincter) botulinum toxinnal való injektálása

TÉRFOGAT

183,7 ml

MÉRÉS IDŐ

18,5 sec

MAX. VIZELÉSI SEBESSÉG VIZELÉSI IDŐ

16,5 ml/s**16,7 sec**

ÁTL. VIZELÉSI SEBESSÉG MAX. SEBESSÉG IDEJE

10,5 ml/s**4,7 sec**

Felső motoneuron sérülés

Alsó motoneuron sérülés

intravesicalis nyomás (H₂Ocm)

Intraabdominalis nyomás (H₂Ocm)

detrusor nyomás (a fentiek különbsége;
(H₂Ocm))

uroflow (ml/sec)

töltőfolyadék mennyisége (ml)

intravesicalis nyomás (H₂Ocm)

intraabdominalis nyomás (H₂Ocm)

detrusor nyomás (a fentiek különbsége;
(H₂Ocm))

uroflow (ml/sec)

töltőfolyadék mennyisége (ml)

uroflow (ml/sec)(zölddel)

töltőfolyadék mennyisége (ml) (kékkel)