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Modulatory effects of intravesical P2X2/3R inhibition on the lower urinary tract electromyographic properties and voiding function of female rats with moderate or severe spinal cord injury

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Abstract

Objectives: To evaluate the role that intravesical P2X2/3 purinergic receptors (P2X2/3R) play in early and advanced neurogenic lower urinary tract (LUT) dysfunction after contusion spinal cord injury (SCI) in female rats.

Materials and Methods: Female Sprague-Dawley rats received a thoracic Th8/Th9 spinal cord contusion with either force of 100 kDynes (kDy; moderate) or 150 kDynes (severe); Sham animals had no injury. Evaluations on urethane-anesthetized rats were at either two or four weeks post-SCI. LUT electrical signals and changes in bladder pressure were simultaneously recorded using cystometry and a set of custom-made flexible microelectrodes before and after intravesical application of the P2X2/3R antagonist AF-353 (10 μ M) to determine the contribution of P2X2/3R-mediated LUT modulation.

Results: Severe SCI significantly increased bladder contraction frequency, and reduced both bladder pressure amplitude and intraluminal-pressure high-frequency oscillations (IPHFO). Intravesical P2X2/3R inhibition did not modify bladder pressure nor IPHFO in Sham and moderate SCI animals, although did increase intercontractile interval. At two weeks after SCI, Sham and moderate-SCI animals presented significant LUT electromyographic activity during voiding, with a noticeable reduction in LUT electrical signals observed at four weeks post-SCI. Intravesical inhibition of P2X2/3R increased the intercontractile interval in Sham and moderate

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SCI rats at either time point, but had no effect on animals with severe SCI. The external urethral sphincter (EUS) showed strong, and P2X2/3R-independent electrical signals in either Sham or moderate-SCI rats in the early SCI stage. At four weeks post-SCI the responsiveness of the EUS was significantly attenuated, independently of SCI intensity.

Conclusions: This study demonstrates that electrophysiological properties of the LUT are progressively impaired depending on SCI intensity and that intravesical P2X2/3R inhibition can attenuate electrical activity in the neurogenic LUT at early, but not at semi-chronic SCI. This translational study should be useful for planning clinical evaluations.

Keywords

Electromyography; cystometry; lower urinary tract; spinal cord injury; P2X3, P2X2 purinergic receptor

INTRODUCTION

Traumatic spinal cord injury (SCI) can interrupt, dependent on the severity, afferent and efferent communication throughout the body. The impaired transfer of information between organs, the spinal cord and the brain results in somatic-motor, autonomic, and sensory deficiencies such as neurogenic bladder dysfunction (1). Consequently, recovery of urinary continence ranks among the top priorities in SCI patients to improve their quality of life (2,3). Cystometric and electromyographic (EMG) assessments are common methods to understand the pathophysiology of neurogenic lower urinary tract (LUT) dysfunction as well as for evaluating changes in bladder function and myoelectric activity in the LUT following SCI (4,5).

Conventional electrophysiology of the external urethral sphincter (EUS) has been widely used to evaluate detrusor-sphincter-dyssynergia (DSD) in animals with SCI (6,7). For instance, motor unit activation patterns in the EUS of an SCI rat can be activated at low, medium or high-pressure thresholds, indicating the loss of LUT synergy and regulatory complications associated with the injury (8). Other studies have provided a working idea of how pelvic innervation regulates activity in the EUS and detrusor contraction during cystometry; therefore unmasking to what extent an SCI could devastate LUT regulation (9,10). In the same sense, a very relevant and recent observation was made that illustrated the ability of LUT afferent pathways to generate hyperexcitable conditions after SCI in rodents. In such study authors found that the high frequency of non-voiding contractions in SCI mice was dependent on activation of C-fiber afferents, with a concomitant relaxation effect on the EUS by the same fibers (4). Furthermore, in a previous study the same group reported that normal EMG activity of the EUS is required for efficient voiding events in either rats or mice with SCI (6). Both studies support the role of dysfunctional sensory innervation in the LUT for promoting erroneous efferent communication that may lead to DSD.

During bladder filling and distension, ATP is primarily released from urothelial cells (11) to activate P2X3 purinergic receptors (particularly P2X2/3₃ and P2X3₃, referred to in this study as P2X2/3R) in afferent nerve terminals which, subsequently initiate the micturition process

(12–14). However, with SCI conditions, a disproportionate release of ATP from urothelial cells exists and may lead to detrusor overactivity (15–17), possibly through a mechanism involving an excessive activation of afferent pathways mediated by purinergic receptors (18,19), predominantly P2X2/3R (20,21).

We determined the effect of P2X2/3R inhibition on the electrophysiological and cystometric responses of the LUT in rats with either moderate or severe SCI (100 or 150 kDynes, respectively). Furthermore, an assessment of progressive LUT functional electrophysiological changes was also characterized at time zero (i.e. Sham group), and at two and four weeks post-SCI. The intravesical infusion of an antagonist specific to (P2X2/3)₃ and (P2X3)₃ receptors (22), allowed us to evaluate the involvement of urothelial P2X2/3R in neurogenic LUT dysfunction (23,24). Our results suggest that SCI-modified electrical nerve signals in the bladder and the EUS could lead to neurogenic LUT and that intravesical inhibition of P2X2/3R may be effective for managing voiding function at early stages following moderate but not severe SCI. Thus, the use of purinergic receptor antagonists should be considered, with some limitations, as an alternative pharmacotherapy for neurogenic LUT symptoms (19,25,26).

MATERIALS AND METHODS

Female Sprague Dawley rats weighing 250–300g were purchased from Envigo (Research Model Services, Houston, TX), housed in a 12h light: 12h dark cycle, with access to food and water *ad libitum*. All experimental procedures were approved by our Institutional Animal Care and Use Committee (IACUC, ID # AUP-0615–0044 / IS00001252), and performed in accordance with the guidelines from the National Institute of Health on the care and use of laboratory animals. In agreement with IACUC requirements, we made every effort to minimize the suffering and number of animals used, while allowing us to obtain meaningful observations.

SCI surgical procedure and experimental groups

A laminectomy was performed at the thoracic Th8/Th9 vertebrae region on each anesthetized animal (2% isoflurane/oxygen), maintaining body temperature with a warm water recirculator set at 37°C throughout all surgical maneuvers. After exposure of the spinal cord tissue, animals were suspended by securing the adjacent Th7 and Th10 vertebral bodies to an Infinite Horizon Impactor (IH-0400, Precision Systems & Instrumentation, Fairfax Station, VA). Using the system software, a spinal cord contusion on the laminectomy region was generated with either a force of 100 kDynes (SCI-100kDy) or 150 kDynes (SCI-150kDy), with a dwell time of one second. Rats from the Sham group were suspended on the impactor for one to two minutes before placing them back on the surgical table. Following the Sham/SCI procedure, the dorsal musculature was sutured, and dermal layers closed with surgical stainless-steel staples (removed after two weeks). The animals were monitored after surgery until fully awake, and visual confirmation of hind leg impairment in rats with SCI. Post-surgical care was equally provided to all animals, and included subcutaneous buprenorphine-XR (0.05 mg/kg; single dose), subcutaneous carprofen (5 mg/kg; twice a day for three days), and intramuscular ampicillin (100 mg/kg; daily for five

days). Rats were housed individually, and urine expelled by manual bladder expression from Sham and SCI-100kD animals for five days. However, animals in the SCI-150kDy groups required manual bladder expression twice daily for a period of 10–14 days after contusion injury. Five separate experimental groups were defined as Sham (2 weeks post-laminectomy; N=6), moderate SCI at 2 weeks (2W-SCI-100kDy; N=6) or 4 weeks (4W-SCI-100kDy; N=6) after contusion injury, and severe SCI at 2 weeks (2W-SCI-150kDy; N=5) or 4 weeks (4W-SCI-150kDy; N=4) post-SCI.

Amplifier system for electrophysiology

Electrical signals in the LUT and changes in bladder pressure were acquired with an RHD-2000 series amplifier (Intan Technologies Inc., Los Angeles, CA) as previously described in intact rats (27). Briefly, electrical signals were recorded with a sampling rate of 5 kHz using a bandwidth varying between 0.09 Hz and 1 kHz. We handcrafted individual flexible microelectrodes that connected the amplifier board directly to five different regions of the rat LUT. Each microelectrode consisted of a 42 AWG micro-coaxial cable (Digi-Key Electronics, Thief River Falls, MN), and one centimeter-long highly flexible biocompatible 316-grade stainless-steel springs of either 0.245 mm outer diameter (OD), or 0.457 mm OD (Motion Dynamics Corporation, Fruitport, MI). Larger electrodes were attached to four different regions of the bladder (bladder dome, upper bladder region, lower bladder region, and bladder base), and to the chest to monitor cardiac signs. To minimize muscle tissue damage, we used a single smaller-OD electrode for recording EUS activity.

Simultaneous cystometric and electrical recordings

Terminal experiments were carried-out in urethane-anesthetized rats (1.2 g/kg; subcutaneously under the dorsal skin of the thorax). This anesthetic was used as cystometric parameters are comparable to those observed in awake animals (28). After reaching a deep anesthetic plane, an abdominal incision was performed to expose the urinary bladder and the pubic bone; the latter was to expose the EUS. At this time, a PE-50 catheter was implanted through the dome of the bladder and secured with 5–0 silk suture, and used for intravesical infusion of vehicle (0.1 % DMSO in 0.9% NaCl) or the P2X2/3R antagonist AF-353 (10 μ M in vehicle solution). After verifying the absence of vehicle leaks at the PE-50 implant area, we placed the 0.457 mm OD electrodes on the described regions of the bladder, while the small 0.254 mm OD microelectrode secured to the mid-region of the EUS. We made an effort to close the abdominal incision as much as possible using 5–0 suture, while taking special care to not disturb the position of the recording microelectrodes, particularly because rats were maintained in a supine position throughout preparation and experimentation. Animals were relocated to an antivibration table covered with a Faraday cage to minimize ambient electromagnetic interference. The suprapubic catheter was connected to an in-line pressure transducer (World Precision Instruments, Sarasota, FL), which was coupled to an auxiliary input on the RHD-2000 amplifier that recorded the signal at rate of 1.25 kHz. Then, all six microelectrodes were connected to the amplifier head-stage. Recordings began after filling the bladder with vehicle to trigger one voiding contraction. Thereafter, either vehicle or AF-353 solutions were infused at a rate of 0.1 ml/min for a period of 20 – 40 min each. Because animals were in a supine position, we deemed voiding contractions by a rise and fall in bladder pressure with visual confirmation of concomitant expulsion of fluid.

Cystometric and electromyographic calculations

The parameters evaluated for cystometric responses included the intercontractile interval (ICI; in seconds), maximal pressure developed during a voiding cycle (in cm H₂O), and duration of intraluminal-pressure high-frequency oscillations (IPHFO; in seconds) during vehicle or AF-353 infusion. Analysis procedures for electrical responses followed those from a previous study (27). Briefly, we employed MATLAB software (MathWorks; Natick, MA) for bandpass filtration at 5 to 20 Hz to reduce breathing and EKG-QRS artifacts, as well as randomized spikes. For peak analysis, the amplitude of electrical signals was calculated throughout the duration of IPHFO in a voiding contraction, or when reaching the peak bladder pressure in the case of the SCI-150kDy animals.

Data analysis

The examples for the simultaneous electrophysiological and cystometric responses shown in Fig.1 and Fig.2 were generated using OriginPro (OriginLab Corp.; Northampton, MA). Statistical differences were determined using unpaired t-tests to compare responses during vehicle vs AF-353 infusion, and ordinary one-way ANOVA with Dunnett's multiple comparison tests against the Sham group during vehicle infusion (both with Prism Software; GraphPad, La Jolla, CA). Values are displayed as mean $\pm$ s.e.m. with $p < 0.05$ considered as statistically significant.

RESULTS

Cystometric LUT responses to SCI and P2X2/3R inhibition.

Cystometric and LUT electrical EMG properties differed between Sham, moderate, and severe SCI animals, as shown in the representative simultaneous EMG and CMG recordings in Figs 1, 2. Moderate injury did not significantly modify cystometric parameters at either two or four weeks. However, when compared to Sham and moderate SCI, the severe injury rats displayed a significant decrease in ICI resulting in an increased voiding frequency, reduced maximal pressure during voiding, and diminished IPHFO duration at both two and four weeks (Figs 1A, 1C, 1D, 2A, 2B, 3A-C). In fact, IPHFO were absent in the 2W-SCI-150kDy. Intravesical inhibition of P2X2/3R increased ICI in Sham and moderately injured animals, although it did not affect the ICI in those animals with severe SCI (150kDy) neither at two weeks nor at four weeks (Fig. 3A; $*p < 0.05$ vs vehicle). Similarly, intravesical perfusion of the P2X2/3R antagonist did not affect maximal voiding pressure during voiding or IPHFO latency (Fig. 3B, C).

EMG responses of the LUT in SCI animals during cystometry and P2X2/3 inhibition.

Bladder and urethral musculature discharged differentially during bladder contraction in Sham and SCI animals (Figs 1A, 1C, 1D, 2A, 2B). Sham animals showed a significant LUT activation during each voiding contraction (Fig. 1A), and the signal amplitude was higher with the appearance of the IPHFO (Fig. 1Aa). The amplitudes of electrical signals recorded in the dome and upper region of bladder were higher than those recorded from the lower region and bladder base (Figs. 4, 5).

When evaluated at two weeks post-SCI, animals with a moderate SCI (100 kDy) presented a response pattern similar to that observed in Sham animals (Fig. 1Cc); on the contrary, rats with a severe SCI (150 kDy) lacked the distinctive LUT electrical response (Fig. 1E). The attenuated electrical response correlated with a dysfunctional voiding pattern where IPHFO were absent and bladder contractions had lower amplitude (Fig. 1Ee) and lower ICI resulting in higher contractile frequency (Fig. 3A). The maximal amplitude of the electrical response observed at the bladder dome of both Sham and 2W-SCI-100kDy animals was essentially absent in the 2W-SCI-150kDy rats (Fig. 4A, $##p<0.01$ vs Sham dome). EUS activity increased in the 2W-SCI-100kDy animals (Fig. 1Cc), but not in the severe injured group (Fig. 1Ee; note differences in scale bars).

Four weeks after SCI, animals with a moderate SCI (100 kDy) have reduced bladder and urethral electrical responses during cystometry (Figs. 2A, A, a, 4A, B). We recorded smaller, yet clearly visible, electrical amplitudes from the LUT in rats with a severe contusion at four weeks following SCI (Fig. 2C). In general, the amplitude at the bladder dome of SCI rats was significantly reduced when compared with the Sham group (Fig. 5A; $\#p<0.05$ for 4W-SCI-100kDy; $##p<0.01$ for 4W-SCI-150kDy). In addition, we recorded the reappearance of brief IPHFO correlating to LUT electrical signals in rats from the 4W-SCI-150 kDy group (Fig. 2Cc).

Intravesical inhibition of P2X2/3R with AF-353 produced a clear reduction of signals in the Sham and 2W-SCI-100kDy groups (Fig. 1B and Bb) but had no further effect on the severely impaired voiding pattern of the 2W-SCI-150kDy animals (Figs. 1F, 1Ff, and 4A; $*p<0.05$ vs vehicle). Although they were of lower amplitude, we observed similar activity patterns in the upper bladder (Fig. 4B; $*p<0.05$ vs vehicle; $##p<0.01$ vs Sham), the lower bladder (Fig. 4C; $*p<0.05$ vs vehicle; $\#p<0.05$ vs Sham), and the bladder base (Fig. 4D; $\#p<0.05$ vs Sham). The amplitude of the electrical response in the EUS was not significantly affected by intravesical inhibition of P2X2/3R in Sham or 2W-SCI-100kDy rats, although it was attenuated in the 2W-SCI-150kDy rat groups (Fig. 4E; $##p<0.01$ vs Sham).

Four weeks after SCI, animals with a moderate SCI (100 kDy) have reduced LUT electrical responses during cystometry (Fig. 2A, Aa), which were again reduced by intravesical inhibition of P2X2/3R (Fig. 2B, Bb). Intravesical application of AF-353 had a lesser effect on the amplitude of the electrical responses, but changed the cystometric characteristics of the 4W-SCI-150kDy rats (Fig. 2C, Cc, D, and Dd). Thus, no significant effects were induced on the amplitude recorded from the dome (Fig. 5A), upper bladder (Fig. 5B), lower bladder (Fig. 5C), or bladder base (Fig. 5D) while infusing the P2X2/3R antagonist. Suppressed electrical activities from the EUS of all SCI animals further show an inhibitory effect by the P2X2/3R antagonist applied intravesically (Fig. 5E; $**p<0.01$ for 4W-SCI-100kDy, and $*p<0.05$ for 4W-SCI-150kDy versus corresponding vehicle condition).

DISCUSSION

Results from this study illustrate how complicated the pathophysiology of the neurogenic LUT after SCI could be. We employed a well-characterized animal model for SCI, which is common to the human condition after an accident (29) and also leads to a range of

locomotor and micturition impairments related to the contusion intensity (30). Our findings show that after either semi-acute (2 weeks) or semi-chronic (4 weeks) post-surgical periods, significant changes to the electrophysiological function of the LUT can be evidenced and evaluated. Electrical signals in the EUS were significantly impaired after four weeks, and intravesical inhibition of P2X2/3R remarkably attenuated EUS responsiveness during voiding contractions. This is very interesting since innervation to the urethra is provided by the pudendal nerve, which has different functions than those from the pelvic or hypogastric nerves, and conveys afferent and efferent information from the EUS to the central nervous system (9, 31). Although the pudendal nerve shares its origin with the pelvic nerve at the L6-S1 spinal segments of the rat (31), it is important to consider that in our SCI model, both the dorsal roots and dorsal horn sensory neurons may be mostly affected by the impact. Results suggest that impaired afferent pathways, which reflexively activate EUS motor neurons during micturition (8), and are key elements leading to neurogenic LUT dysfunction and impaired by a contusion injury independently of the intensity.

Despite the clear differences in locomotor function caused by a moderate SCI (30), we were able to identify a neurogenic LUT dysfunction, by either mild or severe SCI, as early as two weeks after injury. In the case of the SCI-100kDy animals, the neurogenic LUT dysfunction may be associated with an impaired reflexive neural-control at the SCI site triggered by inflammatory processes (30), more than hypertrophy and increased bladder capacity observed in rodents with severe SCI (4). With this experimental approach, pathological variations in cystometric properties and electrical activity of the LUT after SCI can be evaluated, and should allow us to better understand micturition, as well as the participation of multiple voiding sensory pathways affected by SCI (16,33,34). For instance, the feasibility of targeting P2X2/3R in the neurogenic LUT. These receptors are critical for sensory communication to the central nervous system regarding bladder-filling and nociceptive conditions (13,24,35), and our preclinical findings support this information since pharmacological inhibition did reduce LUT EMG activity. However, the fact that this inhibition also increased the intercontractile interval during early SCI stages suggests, as observed in previous studies, that these receptors may be playing a much more complicated role in promoting a neurogenic LUT condition (20,23,36).

The phenotype observed in P2X3-null mice (14,21) can be closely reproduced by pharmacological inhibition of P2X3-formed purinergic receptors (20,27). Indeed, the administration of purinergic P2X3 antagonists has been discussed as a possible treatment for neurogenic bladder dysfunctions where urinary sensory afferents are hyperexcited (37). A preliminary clinical trial evaluated the effects of P2X3 antagonist (AF-219) on patients with confirmed interstitial cystitis (26). The rationale for testing this antagonist was that chronic bladder pain could be associated to an overactivation of P2X3-mediated afferent pathways by excessive release of bladder ATP. In fact, treated patients significantly improved their reported symptoms for bladder pain and urgency. Although the role of ATP release for triggering normal micturition is difficult to evaluate *in vivo*, it is clear that under pathological conditions urothelial ATP may mediate urinary bladder hyperexcitability (13,15). Based on the observation that SCI rats have higher immunoreactivity for urothelial P2X3R (36), it makes it worth determining how effective a chronic intravesical application

of a P2X3R antagonist could be for improving LUT dysfunction. This would save time and resources before initiating a correlated clinical trial.

In this study, we found that during the progression of an average voiding contraction, the bladder dome presents the largest electrical wave amplitude of all LUT electrodes, followed by the EUS, the upper bladder, lower bladder, and then the bladder base. The observation that the dome responds with the largest efferent response may suggest a contraction pattern where the electrical waves originate from that region of the bladder in either normal or neurogenic LUT. The results further suggest that when afferent signaling is attenuated by intravesical P2X2/3R inhibition or an SCI event, the neurogenic dysfunctions manifest as disruptions in the cystometric properties that could be associated with the altered electrophysiological activity of the LUT. Our data support a long-term neurogenic effect that results in affecting not only detrusor performance, but also the responsiveness of the EUS independent of contusion intensity. A dysfunctional EUS effect confirmed in mice with SCI (6).

When comparing ICI values between SCI and Sham rats, a decreased magnitude was inversely proportional to the severity of the injury. Although voiding efficiency/capacity was not calculated, the differences on ICI values may be related to the inability of animals with severe SCI to produce an efficient void, as the urinary bladder does not fully empty and therefore necessarily contracts more often. The intravesical infusion of AF-353 increased ICI in most groups except for animals in the 2W-SCI-150kDy. Thus, inhibition of P2X2/3R could increase the time between effective voiding contractions, and simultaneously decrease detrusor overactivity caused by a defective spinal reflex at the lumbosacral region of animals with SCI (38,39). Similarly, the presence and duration of IPHFO was importantly affected after SCI. Particularly in the SCI-150kDy group, where an elimination of IPHFOs was observed at 2 weeks post-injury, with a sign of minor recovery at 4 weeks. Another cystometric property affected by the severity of the SCI was the maximal pressure developed during a voiding cycle, where, as the severity of the injury increases, the maximal pressure decreases. This impairment may be explained by a detrusor-sphincter-dyssynergia condition typically seen after SCI and manifested as the inability of the EUS to open and close synergistically with the bladder to allow for an efficient voiding event (4, 40).

The electrophysiological responses in animals that sustained a moderate SCI and subsequently evaluated at 2 weeks post injury, closely resembled those of Sham animals although exhibiting a decrease in the amplitude of LUT electrical signals. Conversely, the 4W-SCI-100kDy group demonstrated a clear shift toward reduced electrical activity. Rats from the SCI-150kDy group showed the maximal amount of functional damage, which can logically be associated with the force of the spinal impact affecting sensory and motor regions. Because of the inability of the EUS to trigger normal electrical responses during voiding contractions, these animals develop very inefficient voids resulting in bladder incontinence and deficient compliance. Furthermore, the intravesical inhibition of P2X2/3R suggests that Sham animals, and rats in the early stages of recovery post-moderate SCI, can generate a spinal micturition reflex, perhaps at the lumbosacral region, as well as a brain component that activates efferent and somatic nerves for contraction of the detrusor and EUS activation. In severe SCI or at latter post-moderate SCI stages, this reflex continues to

be dysfunctional leading to impaired LUT function. This hypothesis is supported by the fact that animals with a severe SCI have attenuated electrical responses (i.e. efferent innervation) throughout the whole LUT independent as to when injuries occurred.

Pre-clinical animal models of SCI can provide strategic information before human testing of a specific pharmacological approach; this includes procedures for how to improve the neurogenic LUT. However, our experiments were performed with the animals in a supine position for positioning of recording electrodes, which can affect the normal micturition process. Another comment is that, by surgically separating rectus abdominal muscle, sectioning the pubic bone, and placing multiple electrodes throughout the LUT, this may make the electrophysiological evaluation very invasive. These disadvantages may be addressed by using smaller and more flexible microelectrodes connected to an ambulatory cystometry/EMG recording system on awake animals, as recently reported in other studies (6,41). Nonetheless, the present results have allowed us to elucidate more details about the physiology of micturition, P2X2/3R, and dysfunction of the neurogenic LUT.

It is clear that further experiments and quantitative analysis must be performed to further understand the relationship of electrophysiological properties within the LUT, and the effects that different antagonists can have on them during distinctive recovery stages after the SCI event. One very important and novel observation is the illustration of the devastating effects that a mild SCI has on the electrophysiological and cystometric activity of the LUT in the long term. The presented results suggest that purinergic P2X3R antagonists, applied intravesically, may perhaps help alleviate some of the conditions seen during neurogenic bladder dysfunction without influencing other roles of P2X2/3R throughout the body (19). The current experimental approach would be valuable for alternative evaluations of the physiology of the LUT after SCI, and the characterization of other factors, for example inflammatory factors (42), that lead to neurogenic LUT. Hyperexcitability of spinal sensory neurons during SCI is influenced by activation of P2X2/3R in urothelial cells and bladder afferent nerves by excessive intravesical ATP-release after SCI (15,20). Therefore, the efficacy of P2X3R antagonists for neurogenic and other LUT dysfunctions (43) must be critically addressed using basic science tools before attempting any translational therapy seeking to improve function.

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Figure 1. Electrical and cystometric responses at early post-surgical stage.

Representative examples for simultaneous electrical activity in the LUT (upper traces) and bladder pressure changes (lowest traces) in Sham (A, a); Sham + 10 μ M intravesical AF-353 (B, b); 2W-SCI-100kDy (C, c); 2W-SCI-100kDy + 10 μ M intravesical AF-353 (D, d); 2W-SCI-150kDy (E, e); 2W-SCI-150kDy + 10 μ M intravesical AF-353 (F, f) animals. Arrows point toward the voiding contraction shown in higher magnification next to the main example. Activity from top to bottom corresponds to dome (red line), upper bladder (blue line), lower bladder (magenta line), bladder base (green line), EUS (black line), and bladder pressure (purple line). Scale bars represent 200 μ V in all electrical response traces, and 15 cm H₂O in all cystometric traces. Time scale represent 30 s in A-F, and 2 s in a-f, and applicable to both electrical and cystometric data.

Figure 2. Electrical and cystometric responses at advanced post-SCI stage.

Representative examples for simultaneous electrical activity in the LUT (upper traces) and bladder pressure changes (lower trace) from 4W-SCI-100kDy (A, a); 4W-SCI-100kDy + 10 μ M intravesical AF-353 (B, b); 4W-SCI-150kDy (C, c); 4W-SCI-150kDy + 10 μ M intravesical AF-353 (D, d) rats. Arrows point toward the voiding contraction shown in higher magnification next to the main example. Activity from top to bottom corresponds to dome (red line), upper bladder (blue line), lower bladder (magenta line), bladder base (green line), EUS (black line), and bladder pressure (purple line). Scale bars represent 200 μ V in all electrical response traces, and 15 cm H₂O in all cystometric traces. Time scale represent 30 s in A-D, and 2 s in a-d, and applicable to both electrical and cystometric data.

Figure 3. Cystometric parameters at different time points and SCI intensities.

Changes in intercontractile interval (A), maximal bladder pressure (B), and IPHFO duration (C) during vehicle or 10 μ M AF-353 intravesical infusion in all five experimental groups. Filled bars represent values during cystometry perfusing the vehicle solution, and empty bars the values during application of the P2X3R antagonist. Data represent mean $\pm$ s.e.m. with * p <0.05 vs vehicle infusion within the same group using t-test. # p <0.05 and ## p <0.01 using one-way ANOVA with the Sham condition used as a multiple comparison control. ND indicates not detected.

Figure 4. Group analysis for electrical responses at early post-surgical stages.

Changes in electrical responses during micturition at the bladder dome (A), upper bladder (B), lower bladder (C), bladder base (D), and EUS (E) in Sham, 2W-SCI-100kDy, and 2W-SCI-150kDy groups. Filled bars represent values during vehicle perfusion, while empty bars are the values during application of the P2X3R antagonist. Data represent mean $\pm$ s.e.m. with * $p < 0.05$ vs vehicle infusion within the same group using t-test. Also, # $p < 0.05$ and ## $p < 0.01$ using one-way ANOVA with the Sham condition used as a control for multiple comparisons.

Figure 5. Group analysis for electrical responses at advanced post-SCI stages.

Changes in electrical responses during micturition at the bladder dome (A), upper bladder (B), lower bladder (C), bladder base (D), and EUS (E) in Sham, 4W-SCI-100kDy, and 4W-SCI-150kDy groups. The values from Sham animals shown in Fig. 4 were re-plotted to facilitate comparisons against SCI groups. Filled bars represent values during vehicle perfusion, while empty bars are the values during application of the P2X3R antagonist. Data represent mean $\pm$ s.e.m. with * $p < 0.05$ and ** $p < 0.01$ vs vehicle infusion within the same group using t-test. Also, # $p < 0.05$ and ## $p < 0.01$ using one-way ANOVA with the Sham condition used as a multiple comparison control.