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Developmental and Spinal Cord Injury-Induced Changes in Nitric Oxide-Mediated Inhibition in Rat Urinary Bladder

Debra E. Artim^{1,*}, F. Aura Kullmann², Stephanie L. Daugherty¹, Evan Bupp¹, Cassandra L. Edwards¹, and William C. de Groat¹

¹Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, Pennsylvania

²Urogenix, Inc./Astellas, Durham, North Carolina

Abstract

Aims—During postnatal development large amplitude spontaneous activity of the neonatal rat bladder changes to a low amplitude adult pattern of activity that leads to improved storage function. Previously, we have shown that spontaneous activity in neonatal rat bladder strips is inhibited by activation of the nitric oxide (NO)–cGMP signaling pathway. In the present experiments we determined if this inhibitory pathway is altered during postnatal development or spinal cord injury.

Methods—Baseline tone and amplitude and frequency of spontaneous contractions were measured in bladder strips from male or female neonatal (days 10–21), juvenile (days 24–39) and adult female spinal cord intact or chronic spinal cord injured Sprague–Dawley rats.

Results—The inhibitory effects of an NO donor (SNAP) and a PDE-5 inhibitor (zaprinast) on spontaneous activity of bladder strips decreased during postnatal development, while an inhibitory effect of 8-bromo-cGMP, which was blocked by a protein kinase G inhibitor, was detected at all ages tested. However, the effect of NO–cGMP signaling to reduce baseline tone emerged during postnatal development. The inhibition induced by the NO donor was blocked by an inhibitor of soluble guanylyl cyclase (sGC). Chronic spinal cord injury (cSCI), which causes the re-emergence of a neonatal-like pattern of spontaneous activity, did not restore sensitivity to NO-mediated inhibition in adult rat bladders.

Conclusions—These data indicate that while cGMP signaling inhibits activity in young and adult bladders as well as after cSCI, there is a developmental decrease in the sensitivity of bladder to NO-mediated inhibition.

Keywords

cyclic GMP; phosphodiesterase 5 inhibitors; smooth muscle; spontaneous activity

*Correspondence to: 200 Lothrop St., W1340 Biomedical Science Tower, Pittsburgh, PA 15260. dea20@pitt.edu.

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INTRODUCTION

Previous studies in our laboratory revealed that spontaneous contractile activity in the rat urinary bladder undergoes marked changes during postnatal development^{1,2} and in pathological conditions.³ In vitro whole bladder or bladder strip preparations obtained from neonatal rats during the early postnatal period exhibit large-amplitude, low-frequency rhythmic contractions. During postnatal maturation this activity is eliminated, which improves the storage function of the bladder. However, pathologies such as spinal cord injury⁴ and bladder outlet obstruction³ cause the re-emergence of a neonatal-like pattern of large-amplitude rhythmic contractions. Thus, it has been speculated that pathological conditions in adults may reverse the developmental changes in bladder properties.

The pharmacology of the rat urinary bladder also changes during postnatal development. For example, the neonatal rat bladder (10–21 days) is very sensitive to the inhibitory effect of nitric oxide⁵ and the excitatory effect of L-cysteine.⁶ These effects appear to be markedly diminished during postnatal development as they have not been detected in bladders from adult rats.^{6–8} The developmental change in NO sensitivity could be due to a variety of factors. NO activates soluble guanylyl cyclase (sGC) to produce cyclic guanosine monophosphate (cGMP), which is metabolized by phosphodiesterase 5 (PDE-5). cGMP in turn activates cGMP-dependent protein kinase (PKG), which regulates many cellular processes including smooth muscle relaxation and ion channel function.⁹ Thus, reduced responses to NO in adult bladders could be due to increased PDE-5 expression, decreased sGC expression or decreased expression of the downstream target of cGMP. Developmental regulation of sGC expression patterns and nitric oxide signaling have been reported in other tissues including the heart and pulmonary arteries of the rat^{10–12} and in the chicken ductus arteriosus.¹³

The neural control of the bladder also changes during postnatal development. The neonatal bladder is tonically inhibited by parasympathetic pathways arising in the lumbo-sacral spinal cord.¹⁴ Inhibition of large coordinated rhythmic contractions seen in the neonatal bladder may be necessary early in development to inhibit reflex voiding and allow the mother to control micturition through the perineal-to-bladder excitatory reflex.¹⁵ Later during development, as the bladder improves its storage function, the pattern of spontaneous activity changes along with the development of the mature micturition reflex and the appearance of voluntary voiding.¹⁶ Under these conditions, the inhibitory input may no longer be necessary to achieve continence.

The present study was designed to evaluate the mechanism underlying the developmental change in the sensitivity of intrinsic bladder contractions to modulation by NO signaling. We studied the effects of a nitric oxide donor on activity of bladder strips obtained from juvenile (24–39 days) rats to determine at what age during development the sensitivity to NO is diminished. Additionally, we used bladder strips from chronic spinal cord injured (cSCI) and spinal cord intact adults to determine if cSCI, which causes the re-emergence of a neonatal-like pattern of spontaneous contractile activity, also restores the sensitivity to the inhibitory effects of nitric oxide.

MATERIALS AND METHODS

Bladder Strip Preparation

All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Pittsburgh. Bladder strips from cSCI and spinal cord intact adult female (200–250 g), neonatal (days 10–21) and juvenile (days 24–39) male and female Sprague–Dawley rats were prepared as described previously.² Briefly, the bladder was removed from isoflurane (4% in O₂) anesthetized rats, placed in warm Krebs solution (composition in mM: NaCl 118, KCl 4.7, CaCl₂ 1.9, MgSO₄ 1.2, NaHCO₃ 24.9, KH₂PO₄ 1.2, dextrose 11.7; pH 7.4 bubbled with 95% O₂, 5% CO₂) and cut into four to eight longitudinal strips (~1.5 mm × 8–10 mm) with the urothelium intact. Strips were tied with fine thread at each end, mounted in a vertical double jacketed organ bath in oxygenated Krebs solution (15 ml volume) and kept at 37°C via a circulating warm water bath. Tissue was allowed to equilibrate for 1–2 hr prior to drug testing.

Carbachol (50–100 nM), a muscarinic agonist, was applied to juvenile bladder strips to stabilize the amplitude and frequency of contractions over the course of the experiment. We have used this method previously in neonatal bladder strips,⁵ and showed that carbachol treatment did not alter the inhibitory effect of an NO donor and related drugs. Spontaneous contractions in adult rat bladder strips are stable over the course of an experiment, therefore it was not necessary to add carbachol to these strips. However, to determine if carbachol influenced the effect of drugs in adult strips, in each experiment a proportion of strips was treated with carbachol (50–100 nM) and then with the following chemicals: SNAP (5 out of 12), zaprinast (2 out of 8), and 8-bromo-cGMP (3 out of 14). There were no differences between the effects of these agents on untreated or carbachol treated strips, so the data were pooled. None of the strips from cSCI rats were treated with carbachol. Drugs from concentrated stock solutions were added directly to the organ bath.

After setting baseline tension to 10 mN (1 g) spontaneous and carbachol-enhanced contractions were measured with a force displacement transducer (Grass, Astromed, RI). Data, including baseline tone as well as amplitude and frequency of spontaneous contractions, were recorded for offline analysis using Windaq software (DATAQ Instruments, Inc., Akron, OH) and analyzed using Excel (Microsoft, Redmond, WA) and Origin (version 7; Origin Lab Corporation, Northampton, MA).

Chronic Spinal Cord Injury (cSCI) Model

After a T7–T9 laminectomy under isoflurane anesthesia (2.5% in O₂), the dura and spinal cord were cut at T8 and a sterile sponge (Gelfoam) was placed between the severed ends of the spinal cord. The overlying muscle and skin were sutured. The animals were kept in a room in which the temperature was maintained at 23–25.5°C and were treated with ampicillin (antibiotic) 100 mg/kg, s.c., for 10–14 days, and after 14 days if there was any sign of infection. The bladders of cSCI rats were expressed manually at least two times per day until the return of reflex voiding and then once per day afterwards. Experiments on cSCI rats were performed 3–4 weeks after surgery at which time the bladders were significantly hypertrophied.

Chemicals

Chemicals used in this study include: *S*-nitroso-*N*-acetyl-DL-penicillamine (SNAP), an NO donor, 8-bromoguanosine-3',5'-monophosphate sodium salt (8-bromo-cGMP), a membrane permeable, phosphodiesterase resistant cGMP analog, 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ), an inhibitor of sGC, Rp-8-bromo-PET-cGMPS hydrate (Rp-cGMPS), a cGMP-dependent protein kinase (PKG) inhibitor, zaprinast, a phosphodiesterase (PDE) 5 inhibitor, and carbachol, a cholinergic receptor agonist. Zaprinast, Rp-cGMPS, and 8-bromo-cGMP were obtained from Tocris Bioscience (Ellisville, MO). All other reagents were obtained from Sigma (St. Louis, MO) SNAP was prepared fresh daily in distilled H₂O at 100× final concentration. All other drugs were prepared at 1,000× final concentration and stored at −20°C. Drug stocks were thawed and added directly to the bath to achieve the desired final concentration. Vehicles (0.1% ethanol for ODQ and 0.1% DMSO for zaprinast) had no effect on bladder strip contractions.

Data Analysis

All data are presented as mean ± SEM. Bladder strip activity was measured in 5 min intervals and at the following times after drug application: SNAP 0–5 min, zaprinast 10–15 min, and 8-bromo-cGMP 25–30 min. These measurements were based on our previous experiments using neonatal rat bladder strips,⁵ in which we examined the time–response relationship of the drugs to determine when the maximal effect was produced. The effects of drugs were measured as change in baseline tone or change in amplitude or frequency of contractions that were ≥ 0.1 g. The amplitude of contractions was calculated as the difference between baseline and peak for each contraction. Frequency was determined by manual counting of the number of contractions during the 5 min interval and are presented as contractions/min. An increase in tension was considered a contraction if after the peak, it declined by at least 75% to baseline value. Baseline was measured as the average of 10 minimum points during the 5 min interval. Data are reported as percent of control. Statistical significance was tested with paired two-tailed t-test with layered Bonferroni post hoc test for multiple comparisons when appropriate using Excel and Origin 7 software. Data were considered statistically significant when $P < 0.05$.

RESULTS

Changes in Bladder Smooth Muscle Spontaneous Activity and Sensitivity to Nitric Oxide During Postnatal Development

The amplitude and frequency of spontaneous contractions (in the absence of carbachol) were measured in neonatal (days 10–21), juvenile (days 24–39) and adult rat bladder strips (Fig. 1). The amplitude of spontaneous contractions was not significantly different between neonatal and juvenile bladder strips (Fig. 1A,B); however, the frequency in juvenile strips was significantly higher than in the neonate (4.8 ± 1.1 concontractions/min vs. 3.3 ± 1.2 contractions/min, respectively, $P < 0.05$, Fig. 1A,C). Contractions in adult bladder strips (0.51 ± 0.04 g) were significantly lower ($P < 0.01$) in amplitude compared to contractions (0.84 ± 0.2 g) in juvenile strips (Fig. 1A,B).

As reported previously,⁵ the amplitude and frequency of spontaneous contractions in neonatal bladder strips is significantly inhibited by SNAP (100 μ M), an NO donor (Fig. 2A). On the other hand, in bladder strips from juvenile rats (24–39 days) SNAP (100 μ M) did not significantly decrease the average amplitude and frequency of spontaneous contractions or reduce baseline tone (Fig. 2B,E, $P > 0.05$, $n = 8$). In four of these strips a higher concentration of SNAP (500 μ M) was also ineffective (data not shown). However, there was considerable variability in the effect of SNAP on juvenile bladder strip activity; the effects ranged from 0% to 39% inhibition of contraction amplitude, 2% to 18% reduction of contraction frequency, and 0% to 18% reduction in baseline tone. There was no effect of SNAP in juvenile bladder strips pretreated with the sGC inhibitor ODQ (10 μ M) for 15 min ($n = 12$; $P > 0.05$). SNAP (100 μ M) did not change the amplitude or frequency of spontaneous contractions in any bladder strips from adult rats, but did cause a small reduction in baseline tone (Fig. 2C,F). Pretreatment for 15 min with ODQ (10 μ M), which had no effect alone, prevented the SNAP-induced reduction in the baseline tone (Fig. 2G).

Spinal Cord Injury Causes the Re-Emergence of Neonatal Pattern of Bladder Contractions, But Does Not Restore the Sensitivity to Nitric Oxide

Experiments were conducted in adult rat bladder strips 3–4 weeks after spinal cord injury at which time the bladders were hypertrophied and bladder strips showed increased spontaneous activity compared to strips from spinal cord intact animals (Fig. 1). The amplitude and frequency of spontaneous contractions from cSCI adult bladder strips was similar to that of neonatal rat bladder strips (amplitude 1.6 ± 0.2 g vs. 1.0 ± 0.2 g; frequency 2.2 ± 0.2 contractions/min vs. 3.3 ± 0.4 contractions/min for cSCI and neonates, respectively; Fig. 1B,C). In strips from cSCI rat bladders SNAP (100 μ M) did not significantly affect the amplitude or frequency of spontaneous contractions or baseline tone (Fig. 2D,H).

Sensitivity to cGMP/Is Not Altered During Postnatal Development or Following Spinal Cord Injury

To determine if the decreased sensitivity to NO was due to a decrease in sensitivity to cGMP/PKG signaling, we tested the effects of a membrane-permeable, phosphodiesterase resistant cGMP analog (8-bromo-cGMP, 100 μ M) and a PKG inhibitor (Rp-cGMPs, 25 μ M) on spontaneous activity. At these concentrations, 8-bromo-cGMP significantly suppresses spontaneous activity in neonatal rat bladder strips and the effect is prevented by Rp-cGMPs.⁵ In bladder strips from juvenile rats, application of 8-bromo-cGMP (100 μ M) reduced baseline tone ($19.6 \pm 2.4\%$) and significantly decreased the amplitude ($19.1 \pm 5.3\%$) and frequency ($23.4 \pm 2.7\%$) of spontaneous contractions (Fig. 3A,D). Application of 8-bromo-cGMP (100 μ M) to adult rat bladder strips also markedly decreased the amplitude ($57.2 \pm 8.3\%$) and frequency ($40.8 \pm 10.4\%$) of spontaneous contractions and reduced baseline tone ($30.5 \pm 5.1\%$ reduction). These effects were significantly reduced, but not completely eliminated by 30 min pretreatment with a PKG inhibitor (Rp-cGMPs, 25 μ M; Fig. 3B,E). Rp-cGMPs alone had no effect on any of the parameters of bladder activity. In bladder strips from cSCI rats, 8-bromo-cGMP (100 μ M) also produced a robust inhibition of contraction amplitude ($67.1 \pm 4.4\%$) that was significantly reduced by pretreatment with 25 μ M Rp-cGMPs ($11.9 \pm 10.8\%$ inhibition, $P < 0.05$, Fig. 3F), and had no effect on

contraction frequency (Fig. 3C,F). Baseline tone was also reduced by 8-bromo-cGMP, but this effect was not significantly antagonized by Rp-cGMPs ($28.5 \pm 7.3\%$ reduction in control, $17.6 \pm 5.3\%$ after Rp-cGMPs; $P > 0.05$, Fig. 3F).

Influence of a PDE-5 Inhibitor on SNAP Effects in Juvenile and Adult Bladder Strips

To determine if in older animals the decreased sensitivity to NO was due to increased expression of PDE-5 we tested the effect of the PDE-5 inhibitor zaprinast (25 μ M) on spontaneous activity and its ability to enhance the effects of the NO donor. This concentration of zaprinast has previously been shown to significantly inhibit spontaneous activity and enhance the effect of SNAP in neonatal bladder strips.⁵ Bladder strip activity was measured 5 min before and 10–15 min after zaprinast application, at which time the effect of zaprinast was maximal. SNAP was applied to the bladder strips 5 min after the maximum effect of zaprinast. The effect of SNAP was calculated as the percent change in contractions from measurements of contractions 5 min before and 5 min after SNAP application. In bladder strips from juvenile rats application of zaprinast (25 μ M) significantly reduced the amplitude and frequency of spontaneous contractions and reduced baseline tone within 15 min (Fig. 4A,D). Subsequent application of SNAP (100 μ M) had no further effect on any of the parameters.

In bladder strips from adult rats, zaprinast (25 μ M) produced a small but significant decrease in baseline tension and decreased both the amplitude and frequency of spontaneous contractions (Fig. 4B,E). Application of SNAP after zaprinast produced a large ($37.2 \pm 7.4\%$) decrease in the amplitude of spontaneous contractions, reduced baseline tone but did not alter contraction frequency (Fig. 4B,E). Application of zaprinast to cSCI bladder strips produced only a small decrease in contraction frequency, with no change in contraction amplitude or baseline tone (Fig. 4C,F). Subsequent application of SNAP did not affect spontaneous contractions or baseline tone.

DISCUSSION

The present results indicate that the sensitivity of spontaneous activity in rat bladder strips to an NO donor, but not 8-bromo-cGMP, decreases during postnatal development. The decrease in NO sensitivity is associated with a change from a high-amplitude, low-frequency neonatal pattern of spontaneous activity to a low-amplitude, high-frequency adult pattern consistent with improvement in the storage function of the bladder. cSCI reverses the developmental change in contractile activity, but does not restore the sensitivity to inhibition by NO. The sensitivity to PDE-5 inhibition by zaprinast is also reduced during postnatal development and is not restored in cSCI bladder strips. However, spontaneous contractions in bladder strips from all ages and following cSCI are sensitive to inhibition by 8-bromo-cGMP. Together, these data suggest that NO-mediated inhibition of bladder activity is stronger in the neonate than in the adult. However, the downstream signaling mechanism underlying NO inhibition, which involves activation of PKG, is not altered during postnatal development or following cSCI. Together, these results suggest that the developmental change in NO sensitivity in the rat bladder is due to decreased expression of sGC.

Developmental Changes in Contractile Activity and Sensitivity to NO Signaling

The present data reveal both physiological and pharmacological changes in bladder spontaneous activity during postnatal development. Contraction frequency is increased in juvenile bladder strips, demonstrating a change towards adult pattern of bladder activity. This result is consistent with measurements of spontaneous bladder strip activity from 5-week-old rats using fast Fourier analysis (peak frequency ~0.1 Hz or 6 contractions/min;² vs. this study ~5 contractions/min). In adults, the amplitude of spontaneous contractions decreased, while contraction frequency remained high. Imaging experiments have demonstrated that this high-frequency contractile activity in adult bladders reflects an unorganized pattern of contractions occurring at multiple initiation sites throughout the bladder, whereas the low-frequency high-amplitude neonatal contractions reflect a more organized pattern initiated at one or few sites.⁴

The changes in NO signaling develop gradually during postnatal development and include a decrease in sensitivity to NO and to PDE-5 inhibition. In neonatal rat bladder strips, SNAP (100 μ M) significantly reduces the amplitude and frequency of spontaneous contractions, with no significant effect on baseline tone.⁵ In juvenile rat bladder strips, there was large variability in the effect of SNAP as in some strips it prominently suppressed activity while in other strips it had no effect; the average effect of SNAP was not statistically significant. This suggests a gradual decrease in NO sensitivity over time, which varied in different animals of the same age range. In adult rat bladder strips, SNAP produced a small decrease in baseline tone but had no effect on contraction amplitude or frequency.

Inhibition of PDE-5 with zaprinast also elicited different responses in bladder strips from different age animals. Zaprinast decreased the amplitude and frequency of spontaneous contraction in neonatal,⁵ juvenile and adult strips, but decreased baseline tone only in juvenile and adult strips. In addition, the effects of zaprinast were significantly larger in juvenile than in adult strips. Inhibition of spontaneous activity at all ages by zaprinast suggests that under the conditions of our experiments spontaneous activity can be modulated when PDE-5 is inhibited to allow accumulation of cGMP over time. In the present experiments, the PDE-5 inhibitor had an effect in juvenile and adult strips, even though the effect of SNAP was reduced. Therefore, some basal constitutive cGMP production occurs in the bladder strip preparation.

Application of SNAP after zaprinast significantly inhibited contraction amplitude in adult strips, indicating that sGC is still present and can be activated by NO, although to a lesser extent than in neonatal strips. In juvenile strips however, PDE-5 inhibition did not unmask an NO inhibitory effect as subsequent application of SNAP did not further reduce contractions or baseline tone. This could be explained by the significantly larger effect of zaprinast on contraction amplitude in the juvenile strips which may have been maximal, thereby occluding any effect of subsequent SNAP application. Because the effect of zaprinast decreased with age, it seems reasonable to conclude that the decreased sensitivity of adult rat bladder strips to NO is not mediated by an increase in PDE-5 expression.

Application of 8-bromo-cGMP (100 μ M) reduced the amplitude and frequency of spontaneous contractions and baseline tone in neonatal,⁵ juvenile and adult strips. This

effect was mediated, at least in part, by a PKG-dependent mechanism. This demonstrates that the downstream target of cGMP and PKG is expressed and can be modulated throughout postnatal development and into adulthood.

Possible Mechanisms of Developmental Change in NO Sensitivity

Because the present data argue against a developmental upregulation of PDE-5 expression or decreased expression of the downstream target of cGMP/PKG, a likely explanation for the decreased sensitivity to NO is a developmental decrease or change in sGC expression. Our data showing decreased sensitivity to NO are consistent with several examples of developmental changes in the level and pattern sGC expression in various preparations. In fetal rat pulmonary arteries, sGC β -1 is expressed in smooth muscle cells but not in endothelial cells.¹⁰ During postnatal development sGC expression increases in endothelial cells coupled with a reciprocal decreased expression in smooth muscle. This is thought to underlie the age-dependent differences in the efficacy of inhaled NO treatment of respiratory distress.¹⁰ Similarly, there is a postnatal developmental shift in sGC in the rat heart in which sGC expression is maximal in 10-day-old animals and decreases during development. Cardiac contractility is inhibited by NO in a sGC-dependent manner in neonatal but not adult tissue. Thus, regulation of sGC expression seems to underlie the development changes in NO sensitivity in a variety of preparations. Key future experiments will be to measure sGC expression in the bladder across development and following SCI.

During postnatal development, an inhibitory effect of NO-cGMP signaling on baseline bladder tone appeared and was significant in the adult and the cSCI strips. Baseline tone seems to be very sensitive because it alone is reduced in adult bladder strips by SNAP, and is reduced by 8-bromo-cGMP in juvenile as well as adult strips. Thus, it appears that as sensitivity to NO is reduced during postnatal development, there is the concomitant upregulation of the cGMP pathway inhibiting baseline tone. BK channels are phosphorylated by PKG, leading to increased channel activity which in turn decreases spontaneous activity and tone of bladder smooth muscle.¹⁷ Furthermore, expression of the BK channel in the bladder increases during postnatal development and it is known to contribute to bladder activity and tone.¹ NO may also activate other inhibitory mechanisms. For example, in guinea pig bladder myocytes, an NO donor opens K_{ATP} channels in a cGMP-dependent manner,¹⁸ which can inhibit bladder smooth muscle activity.¹⁹ An additional target of PKG that may underlie the inhibition of spontaneous contractions is Rho kinase, which has been shown to be inactivated by PKG.²⁰ Rho kinase inhibition reduces the peak and sustained components of carbachol-induced contractions in mouse bladder²¹ and decreases bladder tone.²²

Changes in Spontaneous Activity and Sensitivity to NO Signaling Following Spinal Cord Injury (cSCI)

Consistent with previous studies,^{17,23} cSCI resulted in the re-emergence of a neonatal-like pattern of activity characterized by large coordinated rhythmic contractions. Following SCI, the bladders would have been over distended as they were emptied only twice daily, and this would contribute to the generation of detrusor overactivity.²⁴ The pattern of calcium and voltage activity underlying these contractions is better organized than in spinal cord intact

adult rats and is generated from one or two focal sites, in contrast to spontaneous activity in adult bladders that is uncoordinated and initiated from multiple focal sites throughout the bladder.⁴ Spontaneous contractions and baseline tone in cSCI bladder strips are not affected by SNAP. Furthermore, these strips are resistant to PDE-5 inhibition, as only contraction frequency, but not amplitude or baseline tone, is reduced by zaprinast, suggesting that very little endogenous cGMP is being produced in these strips. However, since 8-bromo-cGMP significantly decreased the activity of these strips, the downstream target is still highly sensitive to modulation by cGMP. Therefore, further decreases in sGC may underlie the insensitivity to NO signaling in cSCI bladder strips.

Physiological Relevance

Reduction in the expression of sGC and NO-mediated inhibition of bladder smooth muscle during postnatal development is consistent with the changes in smooth muscle properties and the neural control of the bladder during the postnatal period. As the storage function of the bladder improves during development in concert with the downregulation of large amplitude phasic activity, the necessity for inhibitory mechanisms diminishes and increased efficiency of parasympathetic nerve excitatory mechanisms is required to promote efficient voiding. The expression of NOS in parasympathetic neurons innervating the rat urinary bladder, while considerably lower than in neurons innervating the urethra,²⁵ indicates that these neurons have the potential for releasing NO along with the excitatory neurotransmitters ACh and ATP. Downregulation of sGC in adult bladders would render the bladder smooth muscle resistant to neurally released NO and thereby enhance the excitatory effects of parasympathetic nerves that underlie bladder contractions during micturition.

Clinical Application

Following cSCI NOS expression is increased in the nerves innervating the bladder.²⁶ NO can be released from these nerves and also from the bladder urothelium to act on smooth muscle, interstitial cells or nerves. In addition, the amplitude and number of non-voiding contractions in chronic spinal cord injured rats are decreased by treatment with an arginase inhibitor.²⁷ This effect is prevented by treatment with a NOS inhibitor, demonstrating that the effect of the arginase inhibitor is due to increased NO production.²⁷ Thus, we were expecting that NO signaling may have a greater effect on intrinsic bladder contractile activity after cSCI. However, under the conditions of our experiments, spontaneous activity was very resistant to inhibition by an NO donor or a PDE-5 inhibitor. In contrast spontaneous activity was greatly reduced by the cGMP analog indicating that the downstream target of cGMP/PKG is present and sensitive to modulation.

Clinical studies^{28,29} have demonstrated that PDE-5 inhibitors are effective at reducing male lower urinary tract symptoms (LUTS) and neurogenic bladder dysfunction in cSCI patients,³⁰ and while the rat bladder strip preparation does not model all aspects of cSCI bladder function, these studies indicate that cGMP is produced in the bladder in these conditions and that increasing the levels of cGMP has beneficial effects. Thus, the target of the NO/cGMP signaling that underlies the ability of PDE-5 inhibitors to alleviate LUTS is present in patients, but not in the rat bladder strip preparation. This suggests that the site at which PDE-5 inhibitors act to reduce LUTS is not in the detrusor smooth muscle, but

instead is in the neural pathways controlling the bladder. Enhancing NO inhibition with a PDE-5 inhibitor therefore could decrease the sensory and reflex components of LUTS.

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Fig. 1.

Developmental changes in spontaneous activity in rat bladder strips. **A:** Representative examples of spontaneous bladder strip activity from a neonatal (11 days old) rat, a juvenile (28 days old) rat, an adult rat and a cSCI adult rat 4 weeks post-spinal injury. The neonatal rat bladder strip is characterized by a high-amplitude, low-frequency pattern of spontaneous activity. During development this activity changes to become low-amplitude, high-frequency pattern characteristic of the adult bladder. Spinal cord injury reverses this developmental change causing the re-emergence of a neonatal-like pattern of spontaneous bladder strip activity. Average amplitude (**B**) and frequency (**C**) of spontaneous contractions for each age group. For these measurements, no strips were treated with carbachol. * $P < 0.01$.

Fig. 2.

The effects of SNAP, an NO donor, on amplitude and frequency of spontaneous contractions and on baseline tone change during postnatal development. Examples of SNAP-mediated inhibition of spontaneous activity in neonatal (A), juvenile (B), adult (C), and cSCI adult (D) rat bladder strips. Arrows indicate time of drug application. Calibration bars apply to all traces (A–D). Summary of the effects of SNAP in bladder strips from neonatal (E, hatched bars) juvenile (E, black bars), adult (F), and cSCI (H) rats. G: Summary of the effects of SNAP in adult rat bladder strips in the presence (hatched bar) and absence (solid bar) of ODQ (10 μ M). * P < 0.05 versus control. # P < 0.05 between groups (with and without ODQ).

Fig. 3.

8-Bromo-cGMP inhibits contractile activity and reduces baseline tone in bladder strips of all ages and following spinal cord injury. 8-Bromo-cGMP-induced inhibition of bladder strip spontaneous activity from a juvenile rat (A), an untreated adult rat bladder (B, top trace), an adult rat bladder pretreated with 25 μM Rp-cGMPs (B, bottom trace), an untreated cSCI adult (C, top trace) and a cSCI adult pretreated with 25 μM Rp-cGMPs (C, bottom trace). D: Summary of the effects of 8-bromo-cGMP on the amplitude and frequency of spontaneous contractions and on baseline tone in juvenile rat bladder strips. E,F: Summary of the effects of 8-bromo-cGMP on the amplitude and frequency of spontaneous contractions and on baseline tone in untreated strips (black bars) and in strips pretreated with 25 μM Rp-cGMPs (hatched bars) from an adult rat (E) and from a cSCI adult rat (F) rat. In each experiment, strips from the same bladder were used for Rp-cGMPs treatment and untreated controls. * $P < 0.05$ difference between control and 8-bromo-cGMP. # $P < 0.05$ between 8-bromo-cGMP with and without Rp-cGMPs.

Fig. 4.

Effects of PDE-5 inhibition on spontaneous contractions and on the effect of subsequent application of SNAP, an NO donor. **A:** Example of the effect of zaprinast (top trace) and subsequent application of SNAP (bottom trace) in a juvenile rat bladder strip. **B:** Example of the effect of zaprinast (top trace) and subsequent application (10 min interval) of SNAP (bottom trace) in an adult rat bladder strip. **C:** Spontaneous activity in an cSCI rat bladder strip before (top trace) and after (bottom trace) application of zaprinast. Subsequent application of SNAP had no additional effect. Summary of the effects of zaprinast (black bars) and subsequent application of SNAP (hatched bars) on the amplitude and frequency of spontaneous contractions and baseline tone in bladder strips from juvenile (**D**), adult (**E**), and cSCI adult (**F**) rats. In each experiment, strips from the same bladder were used for zaprinast treatment and untreated controls. * $P < 0.05$ difference between control and zaprinast. # $P < 0.05$ between zaprinast alone and SNAP following zaprinast.