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Correlation of cellular expression with function of NO-sensitive guanylyl cyclase in the murine lower urinary tract

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Key points

- Diseases of the lower urinary tract are associated with dysfunctions of cellular mechanisms that regulate smooth muscle tone. Nitric oxide (NO) mediates relaxation of most smooth muscle-containing tissues via NO-sensitive guanylyl cyclase (NO-GC). Correlation of cellular localization with function of NO-GC in the murine lower urinary tract has not been previously performed.
- Using cell-specific knock-out mice, we demonstrate that NO-GC is expressed exclusively in smooth muscle cells of the urethral sphincter and mediates NO-induced relaxation.
- In bladder detrusor, NO-GC is not detected in smooth muscle cells but rather in platelet-derived growth factor receptor α-positive interstitial cells. NO-GC in these cells does not contribute to NO-induced relaxation; therefore, bladder detrusor smooth muscle appears to be unique as it is not relaxed by NO.
- The correlation of NO-GC localization and function regarding smooth muscle relaxation allows the clinical use of compounds acting within NO/cGMP signalling to be assessed.

Abstract The action of nitric oxide (NO) to stimulate NO-sensitive guanylyl cyclase (NO-GC), followed by production of cGMP, and eventually to cause smooth muscle relaxation is well known. In the lower urinary tract (LUT), in contrast to the cardiovascular system and the gastrointestinal tract, specific localization in combination with function of NO-GC has not been investigated to date. Consequently, little is known about the mechanisms regulating relaxation of the lower urinary tract in general and the role of NO-GC-expressing cells in particular. To study the distribution and function of NO-GC in the murine lower urinary tract, we used internal urethral sphincter and bladder detrusor from global (GCKO) and smooth muscle cell-specific (SM-GCKO) NO-GC knock-out mice for immunohistochemical analyses and organ bath experiments. In urethral sphincter, NO-GC-positive immunofluorescence was confined to smooth muscle cells (SMCs). Deletion of NO-GC in SMCs abolished NO-induced relaxation. In bladder detrusor, exposure to NO did not cause relaxation although immunohistochemistry uncovered the existence of NO-GC in the tissue. In contrast to the urethral sphincter, expression of NO-GC in bladder detrusor was limited to platelet-derived growth factor receptor α (PDGFRα)-positive interstitial cells. In conclusion, NO-GC found in SMCs of the urethral sphincter mediates NO-induced relaxation; bladder detrusor is unique as NO-GC is not expressed in SMCs and, thus, NO does not induce relaxation. Nevertheless, NO-GC expression was found in PDGFRα-positive

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interstitial cells of the murine bladder with an as yet unknown function. Further investigation is needed to clarify the role of NO-GC in the detrusor.

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Abbreviations CCh, carbachol; cGKI, cGMP-dependent protein kinase I; DEA-NO, diethylamine NONOate; FSK, forskolin; GCKO, guanylyl cyclase knock-out; IBMX, 3-isobutyl-1-methylxanthine; ICC, interstitial cells of Cajal; KO, knock-out; LUT, lower urinary tract; LUTS, lower urinary tract symptoms; nNOS, neuronal NO synthase; NO, nitric oxide; NO-GC, nitric oxide-sensitive guanylyl cyclase; PDGFRα, platelet-derived growth factor receptor α; PE, phenylephrine; α-SMA, α-smooth muscle actin; SM, smooth muscle; SMCs, smooth muscle cells; SM-GCKO, smooth muscle cell-specific NO-GC knock-out; WT, wild-type.

Introduction

NO-sensitive guanylyl cyclase (NO-GC) has a key function in nitric oxide (NO)/cGMP signal transduction. As the most important receptor for NO it regulates a large number of physiological processes including smooth muscle relaxation in the cardiovascular and gastrointestinal system, neuronal signal transduction and platelet aggregation (Mellion *et al.* 1981; Moncada & Higgs, 1995; Ignarro, 2002; Friebe & Koesling, 2003; Dangel *et al.* 2010; Groneberg *et al.* 2011).

The lower urinary tract (LUT) consists of the urinary bladder and the urethra. Both tissues contain smooth muscle cells which regulate the tone of the respective tissue and, thereby, contribute to the control of urine storage and micturition. Coordination of contraction and relaxation is needed to guarantee proper filling and voiding. Storage dysfunctions may cause urgency with or without urge incontinence, frequency and nocturia which are the main components of the overactive bladder syndrome (Andersson & Arner, 2004). Dysfunctions concerning voiding include hesitancy, weak stream, sensation of incomplete bladder emptying and post-micturition dribbling. These so-called lower urinary tract symptoms (LUTS) accumulate with increasing age and are a major problem in the older population as they strongly affect quality of life and social functioning (Andersson & Arner, 2004). For that reason it is of great importance to better understand the regulation mechanisms of contraction and relaxation in the LUT.

Several studies have shown involvement of the NO/cGMP pathway in the regulation of smooth muscle tone in the LUT (Persson & Andersson, 1992; Burnett *et al.* 1997; Persson *et al.* 2000). However, to date, the cellular localization of NO-GC has not been described. As a substitute for NO-GC, cGMP immunofluorescence has been used as an indicator for NO/cGMP signalling in urinary bladder tissue (Smet *et al.* 1996; Fujiwara *et al.* 2000; Gillespie *et al.* 2004). Moreover, there is evidence for NO synthase expression in urinary bladder tissue of guinea pigs and humans (Saffrey *et al.* 1994; Smet *et al.* 1996). The functional involvement of NO-GC in the LUT and its association with specific cells, however, has not yet been definitely resolved. Clearly, a combination of both immunohistochemistry and functional experiments is needed to improve our knowledge regarding the involvement of NO-GC in LUT relaxation.

The aim of the present study was to determine the cellular distribution of NO-GC in the murine LUT and to relate NO-GC expression functionally with regulation of smooth muscle tone. In summary, our results clearly show that NO-GC expression in urethral SMCs is responsible for NO-induced relaxation; in the bladder detrusor, NO-GC expression is limited to PDGFRα-positive interstitial cells and is not involved in smooth muscle relaxation.

Methods

Ethical approval

All experiments were conducted in accordance with the German legislation on protection of animals and approved by the local animal care committee.

Animals

Mice (C57BL/6 background) were housed in standard mouse cages (267 mm × 207 mm × 140 mm; maximally 3 animals/cage) with woodchip bedding material and under conventional laboratory conditions (constant room temperature (22°C), humidity level (55%), a 12 h light:12 h dark cycle (lights on at 06.00 h) and either standard rodent diet (wild-type, WT) or fibre-reduced rodent diet (GCKO) (Altromin, Lage, Germany) and water available *ad libitum*). Animals of either sex were killed at age 8–12 weeks (GCKO and WT) or 13–16 weeks (cell-specific KOs and controls) by isoflurane overdose, and tissues were isolated. A total of 65 animals were used.

Generation of GCKO and SM-GCKO mice

Mice lacking NO-GC globally were generated as described previously (Friebe *et al.* 2007). SM-GCKO and interstitial cell of Cajal (ICC)-specific NO-GC knock-out

mice carry a floxed exon (exon 10 of the β_1 subunit of NO-GC) and are transgenic for the inducible Cre recombinase in SMCs (SMMHC-CreER^{T2}; Wirth *et al.* 2008). SM-GCKO mice aged 6–8 weeks were injected with tamoxifen (1 mg I.P.) on 5 consecutive days in order to remove the floxed exon. Fifty days after the last tamoxifen injection, deletion of NO-GC was considered complete (Groneberg *et al.* 2011). Thus, for experiments with SM-specific knock-out tissue, we used animals >50 days after tamoxifen treatment. In every experiment, wherever feasible, WT littermates for GCKO mice or respective heterozygous tamoxifen-injected littermates for cell-specific KO mice were used as controls.

Preparation of murine tissues and isometric force studies

Animals were killed by isoflurane inhalation. The abdomen was opened and the lower urinary tract (LUT) containing urinary bladder and urethra were isolated. The tissues were transferred to Krebs–Henseleit solution (118 mM NaCl, 4.7 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, pH 7.4, 7.5 mM glucose) and bubbled with 95% O_2–5% CO_2.

Urinary bladder detrusor and urethra were separated at the bladder neck. The empty bladder was weighed and the ratio of bladder weight/body weight was calculated. The bladder detrusor was then cut into four strips which

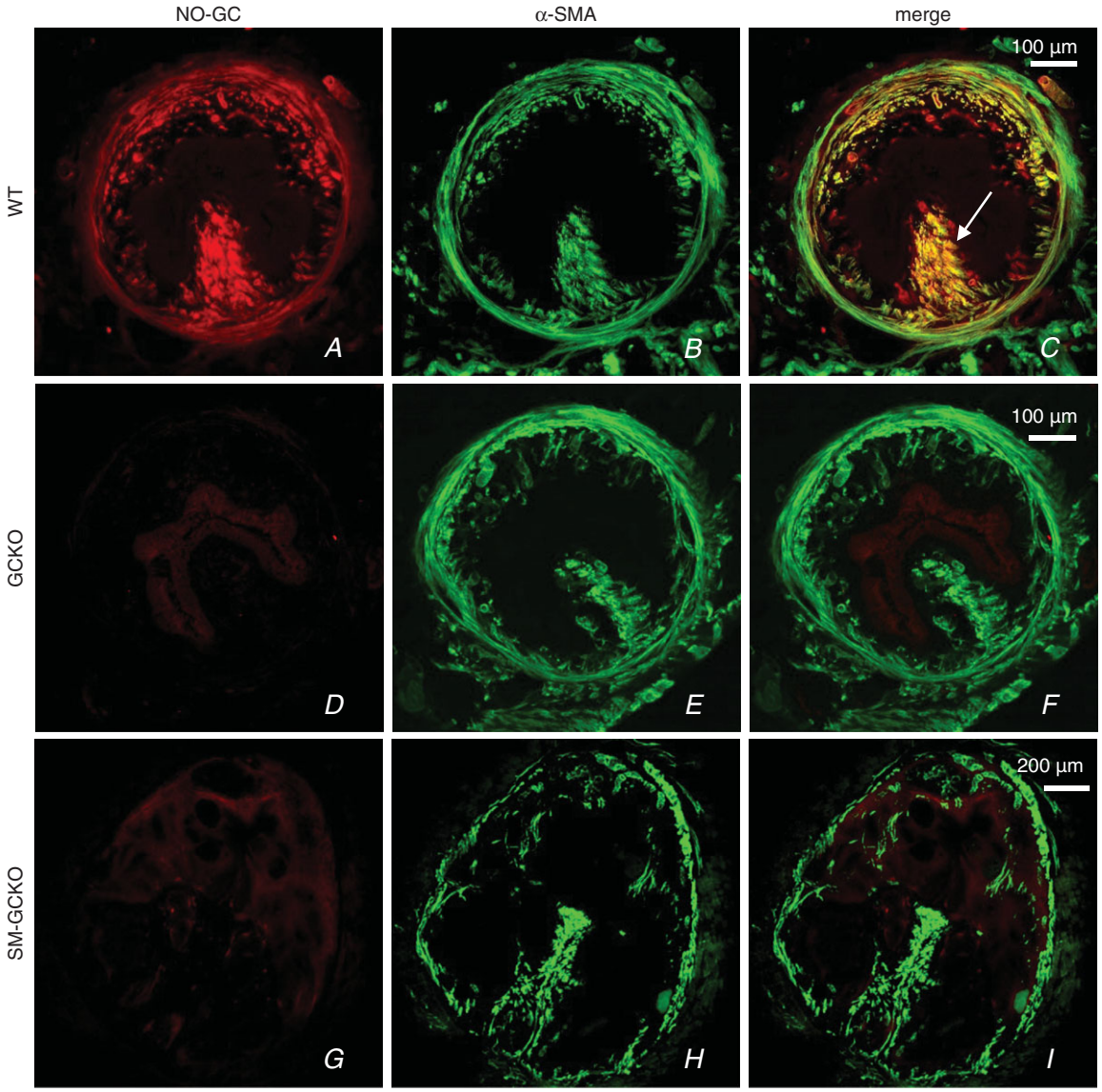

Figure 1. Immunohistochemical localization of NO-GC in internal urethral sphincter
Urethral tissues from WT, GCKO and SM-GCKO were stained with specific antibodies for NO-GC (*A*, *D*, *G*) and α-SMA (*B*, *E*, *H*). The merge (*C*, *F*, *I*) shows localization of NO-GC in smooth muscle cells. Arrow indicates the uvula of the bladder.

were mounted longitudinally onto fixed segment support pins in two four-chamber myographs (Myograph 610, Danish Myo Technology, Denmark) containing 5 ml of Krebs–Henseleit solution bubbled with 95% O_2–5% CO_2. Resting tension was set to 5 mN. After equilibration (at least 60 min at 37°C), strips were pre-contracted with carbachol (CCh; 0.1 μM) and the relaxing effects of 2-(*N*,*N*-diethylamino)-diazenolate-2-oxide.diethylammonium salt (DEA-NO), *para*-chlorophenylthioguanosine-3′,5′-cyclic monophosphate (8-pCPT-cGMP) or forskolin were determined. 3-Isobutyl-1-methylxanthine (IBMX, 100 μM) was added at the end of each experiment to determine maximal relaxation.

Urethral experiments were performed in accordance with bladder detrusor experiments. Proximal urethra, including the internal urethral sphincter, was isolated by removing the connective tissue. Urethral sphincter rings were mounted on the myograph and resting tension was set to 8 mN. After equilibration, urethral sphincters were pre-contracted with phenylephrine (PE, 0.1 μM). Relaxation was induced with DEA-NO, 8-pCPT-cGMP or forskolin as indicated. IBMX (100 μM) was used as in bladder detrusor.

Immunohistochemistry

Mice were killed by inhalation of an overdose of isoflurane and tissue was snap frozen using liquid nitrogen. Cryosections (50 μm for urethra, 20 μm for bladder detrusor) were cut, air-dried and incubated overnight with a homemade antibody against the β_1 subunit of NO-GC raised in rabbit (1:800; Friebe *et al.* 2007) together with a fluorescein isothiocyanate (FITC)-conjugated mouse anti-α-smooth muscle actin antibody (1:500, clone 1A4, Sigma-Aldrich, Munich, Germany), a rat anti-platelet-derived growth factor receptor α (PDGFRα) antibody (1:200, clone APA5, eBioscience, Frankfurt, Germany) or a rat anti-ckit antibody (1:400, clone ACK4, Linaris, Wertheim-Bettingen, Germany). The rabbit antibody was detected with an Alexa 555-conjugated anti-rabbit IgG antibody raised in donkey (1:800, Invitrogen, Darmstadt, Germany); the rat antibodies were detected with an Alexa 488-conjugated donkey anti-rat IgG antibody (1:800, Invitrogen, Darmstadt, Germany) for 1 h. The sections were mounted in Mowiol and were evaluated using a confocal microscope (Leica TCS Sp5).

Individual statistical analyses

For calculation of statistical tests, GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA, was used. Mann–Whitney *U* tests were used for the data in Figs 2, 3 and 6 in a predefined sequence. The results for bladder weight/body weight ratio are expressed

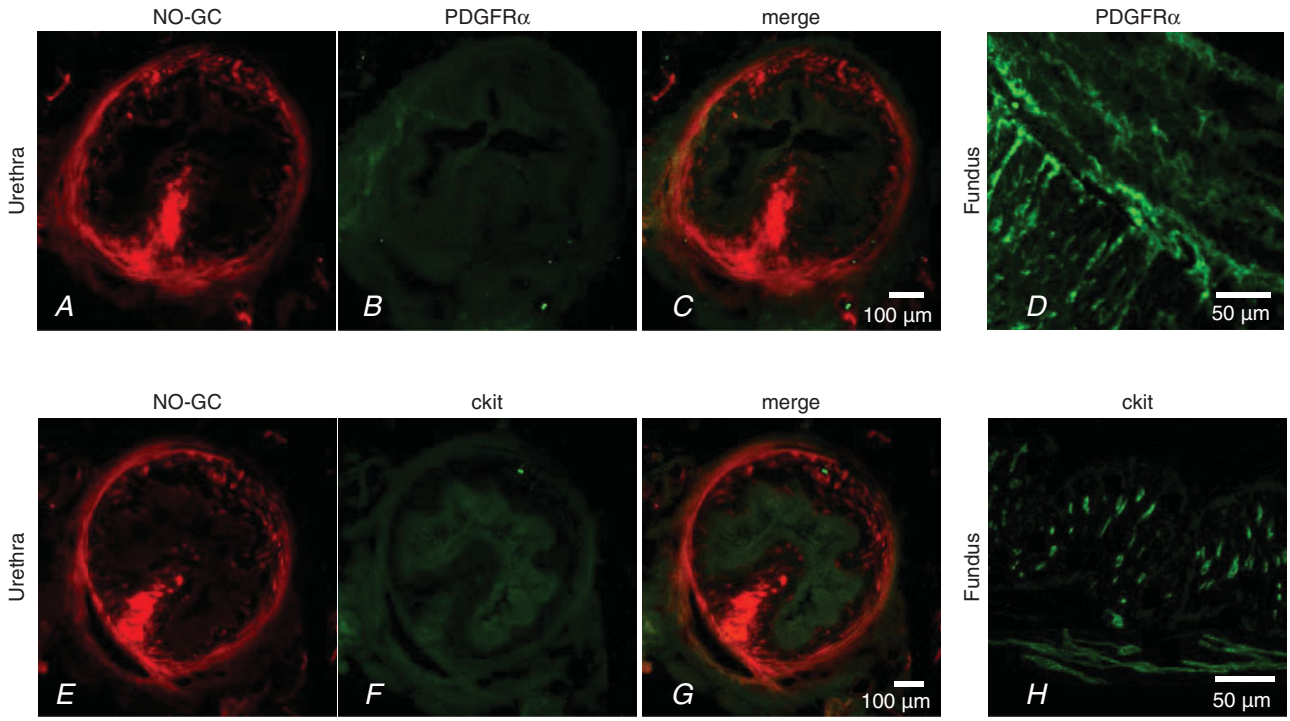

Figure 2. Absence of ckit- and PDGFRα-positive staining in internal urethral sphincter
Urethral tissues from WT were stained with specific antibodies for NO-GC (*A*, *E*) and PDGFRα (*B*) or ckit (*F*). The merges are shown in *C* and *G*. Fundus tissue treated identically to urethra was used as positive control (*D*, PDGFRα; *H*, ckit).

as mean ± SD. All other data are expressed as mean ± SEM (n = number of animals).

Materials

DEA-NO, was obtained from Alexis Biochemicals (Lausen, Switzerland). 8-pCPT-cGMP was obtained from BioLog Life Science Institute (Bremen, Germany). CCh, IBMX and PE were purchased from Sigma (Taufkirchen, Gemany). Forskolin was purchased from Tocris Bioscience (Wiesbaden-Nordenstadt, Germany).

Results

Immunohistochemical analysis of NO-GC expression in urethral sphincter

First, we performed immunohistochemistry to identify NO-GC-expressing cells in the murine internal urethral sphincter. Figure 1*A–C* shows co-localization of NO-GC and α-smooth muscle actin (α-SMA) immunoreactivity indicating NO-GC expression in SMCs of WT urethra. As expected, GCKO animals did not express NO-GC (Fig. 1*D–F*). Similar to GCKO, staining of SM-GCKO tissue (Fig. 1*G–I*) revealed the absence of NO-GC expression. Taken together, this set of data indicates that in the urethral sphincter NO-GC is only expressed in SMCs. Antibody staining to the receptor tyrosine kinase ckit, the gold standard for labelling ICC in the gastrointestinal tract, did not yield any signal above background levels in murine urethra (Fig. 2*A–D*). Similarly, no signals were detected using an antibody specific against platelet-derived growth factor receptor α (PDGFRα), a marker for fibroblast-like cells in the gastrointestinal tract (Fig. 2*E–H*; see below).

Relaxation of the internal urethral sphincter

Using myography, we then tested NO-induced relaxation in pre-contracted (0.1 μM PE) urethral sphincters. The NO donor DEA-NO led to a concentration-dependent relaxation of urethral sphincters from WT mice which

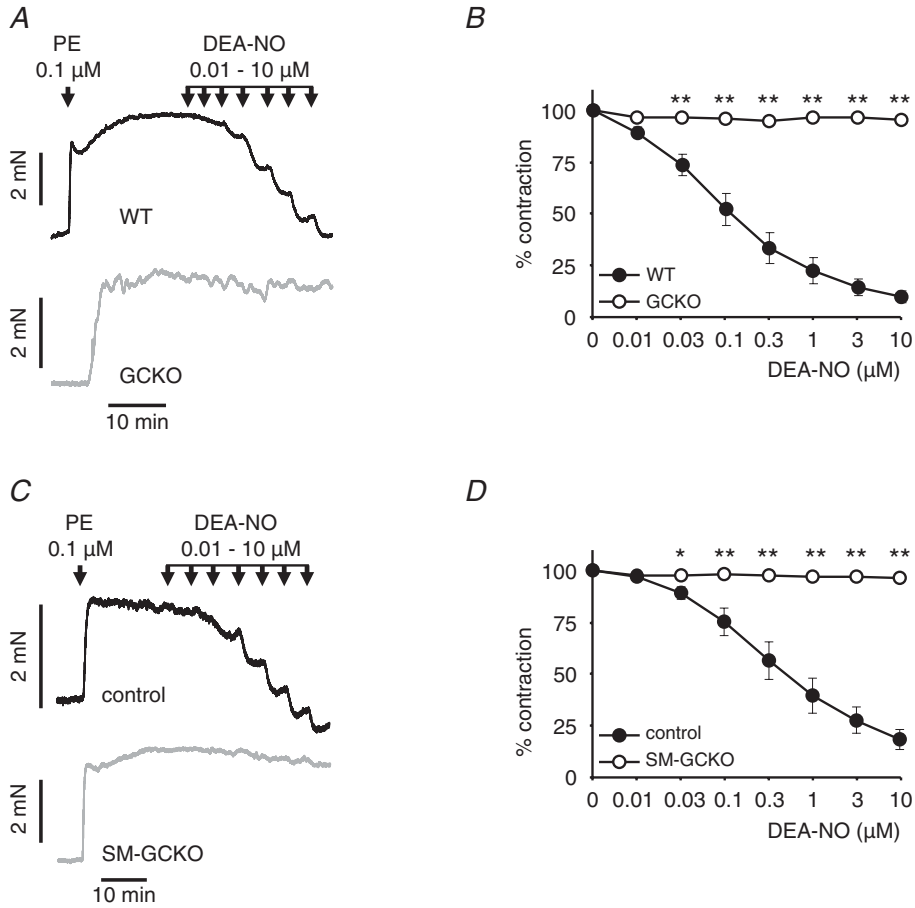

Figure 3. DEA-NO-induced relaxation of urethral rings
DEA-NO-induced relaxation of pre-contracted (PE; 0.1 μM) urethral rings from GCKO and WT. *A*, original trace; *B*, statistical analysis (**$P < 0.01$). Data shown are mean ± SEM of $n = 6$ per genotype. DEA-NO-induced relaxation of pre-contracted (PE; 0.1 μM) urethral rings from SM-GCKO and respective control animals. *C*, original trace; *D*, statistical analysis (*$P = 0.0159$, **$P < 0.01$). Data shown are mean ± SEM of $n = 5$ per genotype.

was abrogated in GCKO mice (Fig. 3*A* and *B*). This clearly indicates NO-GC to be the main mediator of NO-induced relaxation in murine urethral sphincter. NO-induced relaxation was also abolished in urethral sphincter from SM-GCKO (Fig. 3*C* and *D*), which is in accordance with the lack of NO-GC expression in SMCs (see Fig. 1*G–I*).

In the gastrointestinal tract, ICC have been shown to mediate nitrergic relaxation (Sanders *et al.* 2010; Groneberg *et al.* 2011). However, urethral sphincters from ICC-specific GCKO animals showed a WT-like relaxation to DEA-NO (not shown) which indicates that NO exclusively acts via NO-GC in SMCs of the urethral sphincter arguing against an involvement of ckit-positive interstitial cells.

To find out whether the NO/cGMP cascade downstream of NO-GC was still intact in KO tissue, we applied a bolus of 10 μM 8-pCPT-cGMP to urethral sphincters from WT, GCKO and SM-GCKO mice. No significant differences were observed between the different mouse strains indicating a preserved downstream pathway (Fig. 4*A*). In addition, we used forskolin as an activator of adenylyl cyclase to monitor cAMP-induced relaxation. Figure 4*B* and *C* shows that there are no differences in cAMP-mediated relaxation between urethral sphincters from WT, GCKO and SM-GCKO animals.

Immunohistochemical analysis of urinary bladder detrusor

Bladder detrusor from WT mice showed intense staining for NO-GC (Fig. 5*A*), which was absent in GCKO animals (Fig. 5*D–F*). Surprisingly, co-staining of NO-GC with α-SMA in WT bladder tissue did not indicate NO-GC expression in SMCs; instead, NO-GC staining was found in between the smooth muscle bundles (Fig. 5*A–C*). Accordingly, the NO-GC staining was not affected by SMC-specific deletion indicating NO-GC expression in non-SMCs of bladder detrusor (Fig. 5*G–I*). Using an antibody against platelet-derived growth factor receptor α (PDGFRα), a marker for fibroblast-like cells in the gastrointestinal tract and also recently described in bladder detrusor (Koh *et al.* 2012), we found distinct expression of NO-GC in PDGFRα-positive cells in WT bladder detrusor (Fig. 6*A–C*).

Size of the urinary bladder

Bladder weight was not affected by NO-GC deletion (bladder weight/body weight ratio: 1.3 ± 0.3 mg g^{-1} (WT; $n = 26$); 1.4 ± 0.5 mg g^{-1} (GCKO; $n = 19$); n.s., $P = 0.1142$) arguing against dysfunctional mechanisms leading to smooth muscle remodelling or hypertrophy. This is consistent with the results found in mice lacking cGMP-dependent protein kinase I (cGKI; Persson *et al.* 2000) but contrasts the findings from mice deficient in neuronal NO synthase (nNOS; Burnett *et al.* 1997). Also, we did not detect any difference in bladder weight/body weight ratio between male and female mice.

Relaxation of urinary bladder detrusor

As in urethra, we evaluated the impact of DEA-NO on bladder detrusor. Since cholinergic mechanisms are known to mediate contraction in bladder detrusor

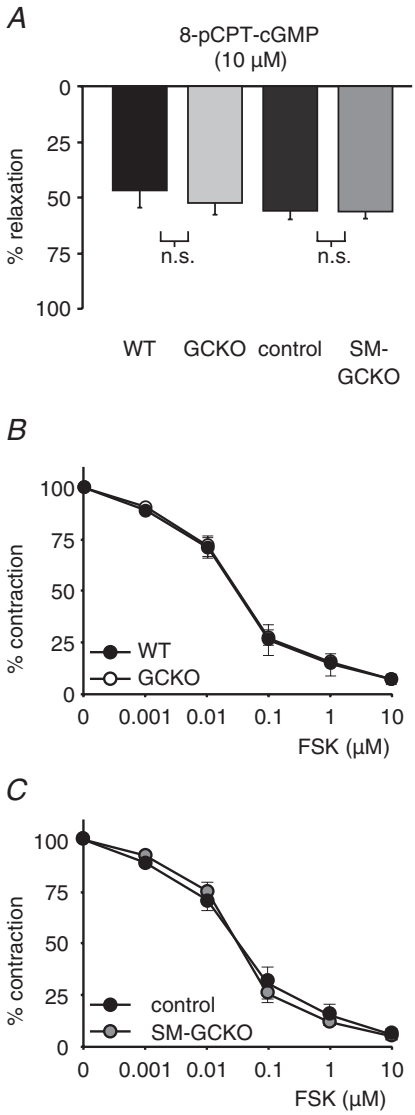

Figure 4. Urethral relaxation to 8-pCPT-cGMP and forskolin
A, 8-pCPT-cGMP-induced relaxation of urethral rings from GCKO and WT animals (n.s., $P = 0.6905$) as well as SM-GCKO and respective control animals (n.s., $P = 0.8413$). *B*, forskolin (FSK)-induced relaxation of urethral rings from GCKO and WT animals (n.s., $P = 0.6905$ to 1). Data shown are mean ± SEM of $n = 5$ per genotype. *C*, forskolin-induced relaxation of urethral rings from SM-GCKO and control animals (n.s., $P = 0.4857$ to 1). Data shown are mean ± SEM of $n = 4$ per genotype.

(Andersson & Arner, 2004), pre-contraction of bladder strips was induced with 0.1 μM CCh. In contrast to urethral tissue, even high concentrations of the NO donor did not relax bladder detrusor from GCKO or WT animals (Fig. 7*A*). We also tested the reaction of bladder detrusor to 8-pCPT-cGMP. Application of 8-pCPT-cGMP caused hardly any relaxation in either GCKO or WT bladder strips (Fig. 7*B*), which corroborates the results seen with DEA-NO. Application of forskolin, though, led to a concentration-dependent relaxation suggesting a more important role of the cAMP cascade in bladder detrusor relaxation (Fig. 7*C*). The fact that forskolin relaxes detrusor from GCKO and WT animals equally well indicates that the cAMP pathway was not affected by the NO-GC deletion.

To summarize, NO/cGMP-mediated relaxation is of importance in the internal urethral sphincter but not in bladder detrusor. We are the first to localize NO-GC in PDGFRα-positive cells in the murine bladder detrusor. However, the function of NO-GC in these cells remains to be elucidated.

Discussion

Lower urinary tract symptoms (LUTS) are becoming increasingly prevalent in elderly people of both sexes.

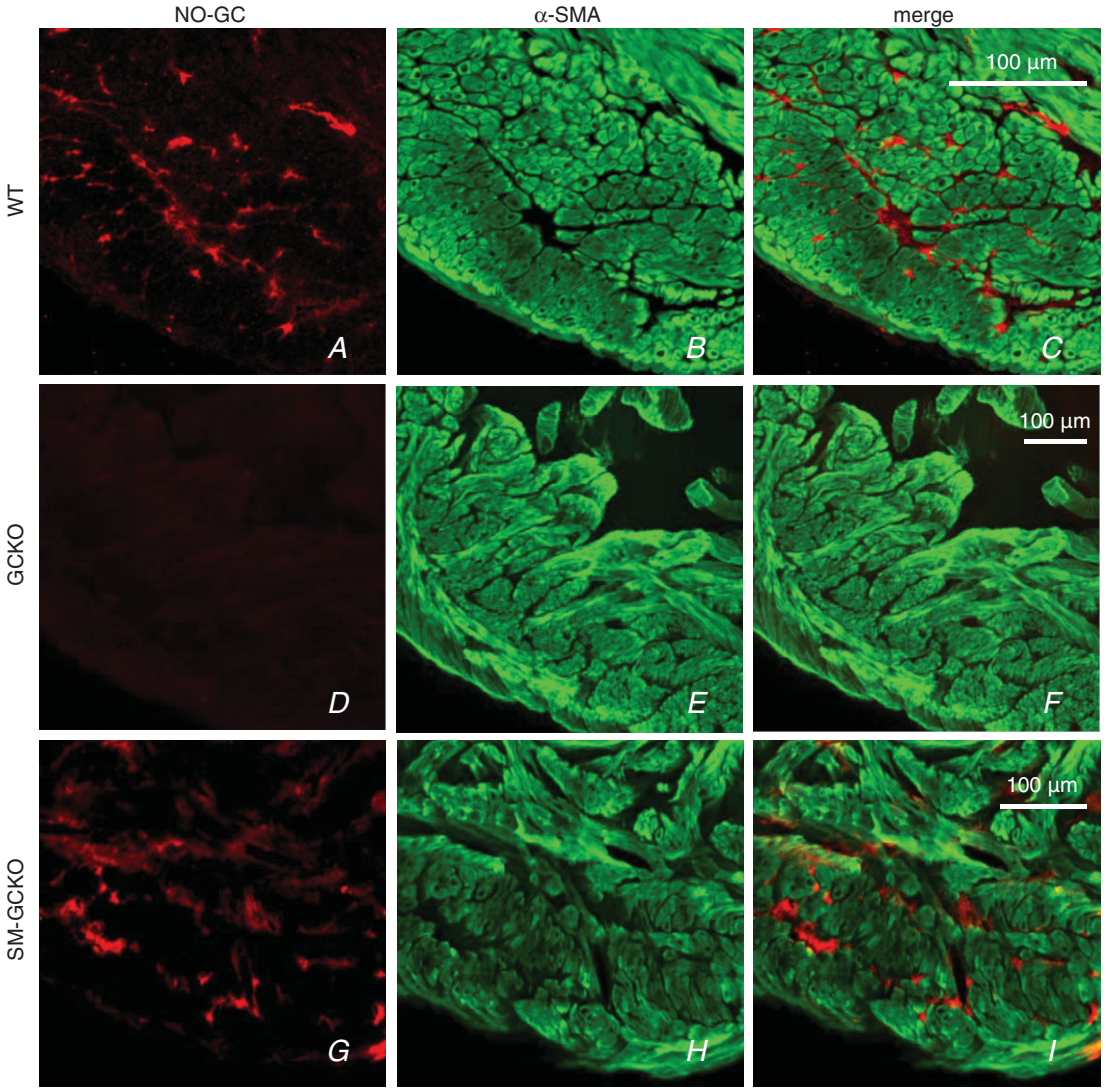

Figure 5. Immunohistochemical localization of NO-GC in bladder detrusor
Bladder detrusor was stained with specific antibodies for NO-GC (*A*, *D*, *G*) and α-SMA (*B*, *E*, *H*). The merge (*C*, *F*, *I*) shows localization of NO-GC in between smooth muscle bundles.

The occurrence of LUTS in males has been studied intensively since there is a correlation between LUTS and benign prostatic obstruction frequently occurring in older age (Takeda *et al.* 2003). Although there are only a few studies that examine LUTS beyond incontinence in women, LUTS have been shown to be prevalent in females as well (Swithinbank *et al.* 1999). Due to the ageing population, increasing numbers of people suffer from the consequences of this illness. This emphasizes the importance of investigating aetiology and, generally, understanding LUT (patho-)physiology to lay the ground for improving therapeutic treatments.

To date, information about the nitrergic influence on contraction and relaxation patterns in both urinary bladder and urethra is still insufficient. In the murine LUT, to the best of our knowledge, expression of NO-GC has not been studied before. Therefore, in the present study, we sought to correlate the cellular expression of NO-GC with the regulation of SM tone in the murine LUT using global and cell-specific knock-out mice.

NO-induced relaxation was seen in urethral sphincters in WT but was absent in tissues from GCKO and SM-GCKO mice. This clearly supports the assumption that NO-GC in SMCs mediates urethral sphincter relaxation. Our immunohistochemical analyses corroborate this notion as NO-GC expression was only detected in SMCs of urethral sphincter. There is no information on NOS expression in the murine urethra although nNOS was detected by immunohistochemistry in nerve fibres of other species, e.g. rabbit, guinea pig and humans (Smet *et al.* 1996; Waldeck *et al.* 1998). The participation of nitrergic mechanisms in urethral relaxation has been shown in several studies in rat and mouse (Fujiwara *et al.* 2000; Triguero *et al.* 2009). In addition, studies with nNOS and cGKI knock-out mice show a reduced urethral relaxation to NO donors and neuronally released NO thus supporting a participation of the NO/cGMP pathway in murine urethral relaxation (Burnett *et al.* 1997; Persson *et al.* 2000). Yet, due to the lack of cell-specific cGKI/NOS knock-out animals, the identity of the cells mediating NO/cGMP signalling has not been studied in detail.

As the deletion of nNOS (but not of cGKI) was shown to result in an increased bladder weight, we measured the bladder weight/body weight ratio in our mice. Bladder weight/body weight ratio of GCKO animals did not differ from that of WT mice; we can thus rule out hypertrophy or other macroscopic changes. A similarly unchanged bladder weight/body weight ratio was published for cGKI knock-out mice (Persson *et al.* 2000). Moreover, we did not notice any difference between female and male bladder weight/body weight ratio in our study; this again is in line with the results obtained with cGKI knock-out mice but inconsistent with those from nNOS knock-out animals. The divergence between nNOS knock-out results and the results found in cGKI and NO-GC knock-out animals may be due to the fact that the bladder weights of nNOS KO mice were not normalized (Burnett *et al.* 1997).

The role of interstitial cells in murine urinary bladder and their general participation in NO/cGMP signalling is still unclear. Interstitial cells appear to be localized in the lamina propria, intramuscular, between muscle bundles and perivascular (McHale *et al.* 2006; McCloskey, 2013). The issue is, however, complicated as anti-ckit antibodies only label a subgroup of interstitial cells in the bladder. ckit-positive interstitial cells have been shown in guinea pig and human bladder tissue (McCloskey & Gurney, 2002; Shafik *et al.* 2004). In murine urinary bladder McCloskey *et al.* (2009) found ckit-positive cells whereas Pezzone *et al.* (2003) and our group could not confirm these results. cGMP production has been detected in as yet uncharacterized interstitial cells of guinea pig (Smet *et al.* 1996; Gillespie *et al.* 2004) and murine urinary bladder (Fujiwara *et al.* 2000).

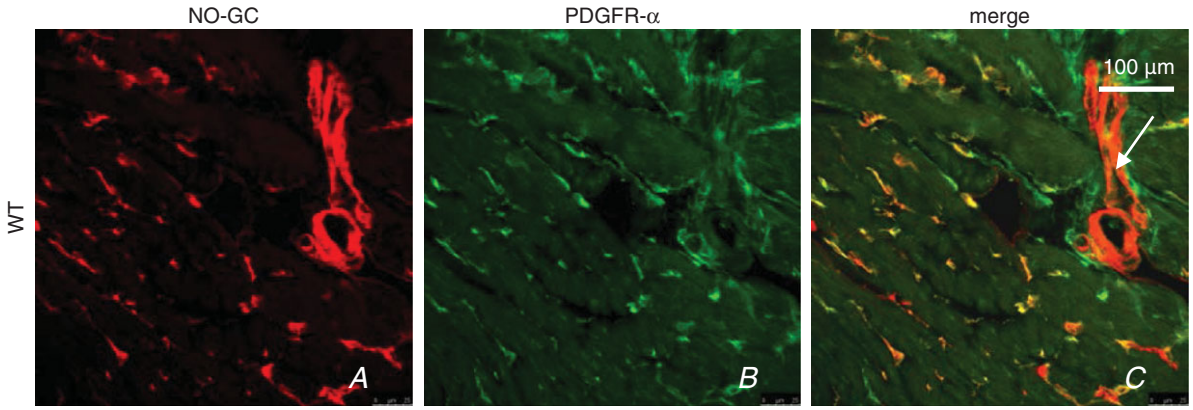

Figure 6. Immunohistochemical localization of NO-GC in PDGFRα-positive cells of bladder detrusor
Bladder detrusor was stained with specific antibodies for NO-GC (*A*) and PDGFRα (*B*). The merge (*C*) shows the co-localization of both immunosignals in the interstitial cells between the smooth muscle bundles. The arrow indicates a blood vessel with vascular SMCs positive for NO-GC.

In our study we examined the expression of NO-GC as the key enzyme in the NO/cGMP pathway in bladder detrusor. We could distinctly localize NO-GC to interstitial cells between SM bundles. Co-staining with PDGFRα, a marker used for fibroblast-like cells in the GI tract, showed a clear co-localization implying NO-GC expression in PDGFRα-positive cells. These interstitial cells have been recently demonstrated to exist in the bladder detrusor by Koh *et al.* (2012). Although we did not stain for cGMP, the existence of NO-GC in PDGFRα-positive cells suggests that the cGMP-positive interstitial cells/stromal cells found by Smet *et al.* (1996) and Fujiwara *et al.* (2000), respectively, may well be PDGFRα-positive interstitial cells. To our surprise, even though we obtained NO-GC expression, bladder detrusor lacked relaxation to DEA-NO or the cGMP-analogue 8-pCPT-cGMP. This may be explained by the fact that NO-GC is expressed in interstitial cells rather than in SMCs. Although PDGFRα-positive cells in the gastrointestinal tract are likely to be involved in purinergic neurotransmission (Kurahashi *et al.* 2011), their function in the LUT and the role of NO-GC in these cells are as yet unknown.

Taken together, our data uniquely demonstrate that specific deletion of NO-GC in SMCs abolishes the

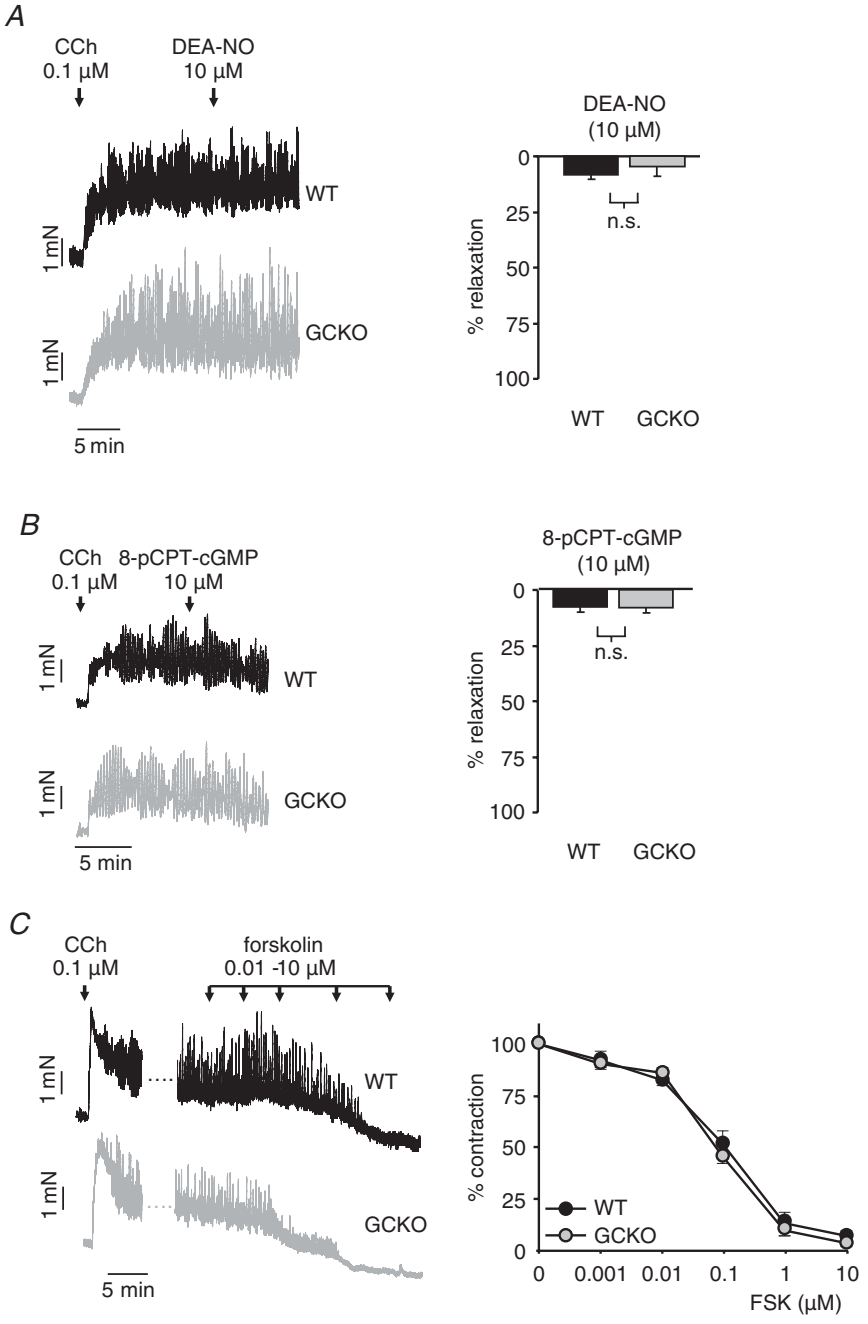

Figure 7. Effects of DEA-NO, 8-pCPT-cGMP and forskolin on bladder detrusor tone
Detrusor muscles from WT and GCKO mice were pre-contracted with CCh (0.1 μM). Left panels show original traces and right panels the statistical analysis. Neither DEA-NO (*A*; 10 μM; n.s., $P = 0.1061$) nor 8-pCPT-cGMP (*B*; 10 μM; n.s., $P = 0.7546$) had a significant relaxing effect on either genotype. Forskolin (FSK) induced a concentration-dependent relaxation of detrusor from both WT and GCKO mice (*C*; n.s., $P = 0.2500$ to 1). Data shown are mean ± SEM of $n = 5$ (WT) and $n = 3$ (GCKO).

NO/cGMP-dependent relaxation of murine urethral sphincter. Thus, NO-GC in SMCs is the only effector to mediate NO-induced relaxation of the urethral sphincter. In bladder detrusor, however, the NO/cGMP pathway seems not to be involved in relaxation, which is in contrast to all other smooth muscle tissues being relaxed by NO; rather, cAMP may play a crucial role. Nevertheless, NO-GC is abundantly expressed in PDGFRα-positive cells with an as yet unknown function. Its participation in regulating bladder functions will be subject to further studies.

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Additional information

Competing interests

None declared.

Author contributions

Conception and design of the experiments: B.L. and A.F.; collection, analysis and interpretation of data: B.L., D.G. and A.F.; drafting the article or revising it critically for important intellectual content: B.L. and A.F. All authors have read and approved the final submission.

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