Functional characteristics of urinary tract smooth muscles in mice lacking cGMP protein kinase type I

KATARINA PERSSSON, RAJ KUMAR PANDITA, ATTILA ASZÖDI, MARIANNE AHMAD, ALEXANDER PFEIFER, REINHARD FÄSSLER, AND KARL-ERIK ANDERSSON. Functional characteristics of urinary tract smooth muscles in mice lacking cGMP protein kinase type I. Am J Physiol Regulatory Integrative Comp Physiol 279: R1112–R1120, 2000.—Nitric oxide (NO)-mediated smooth muscle relaxation is mediated by cGMP through activation of cGMP-dependent protein kinase I (cGKI). We studied the importance of cGKI for lower urinary tract function in mice lacking the gene for cGKI (cGKI−/−) and in litter-matched wild-type mice (cGKI+/+) in vitro and in vivo. cGKI deficiency did not result in any changes in bladder gross morphology or weight. Urethral strips from cGKI−/− mice showed an impaired relaxant response to nerve-derived NO. The cGMP analog 8-bromo-cGMP (8-BrcGMP) and the NO-donor SIN-1 relaxed the wild-type urethra (50–60%) but had only marginal effects in the cGKI-deficient urethra. Bladder strips from cGKI−/− mice responded normally to electrical field stimulation and to carbachol but not to 8-BrcGMP. In vivo, the cGKI-deficient mice showed bladder hyperactivity characterized by decreased intercontraction intervals and nonvoiding bladder contractions. Loss of cGKI abolishes NO-cGMP-dependent relaxations of urethral smooth muscle and results in hypertensive voiding. These data suggest that certain voiding disturbances may be associated with impaired NO-cGKI signaling.

Inhibitory nonadrenergic, noncholinergic (NANC) neurotransmission has been demonstrated in lower urinary tract smooth muscles from different species, and evidence has accumulated that L-arginine-derived nitric oxide (NO) is the main mediator of NANC effects (2, 6). NO effectively relaxes isolated smooth muscle preparations from the outflow region of various species, including humans, suggesting that NO may be involved in the decrease in intraurethral pressure observed at the start of normal micturition. Inhibitors of NO synthase (NOS) cause voiding abnormalities in rats (4, 27) and fetal sheep (23). Mice with targeted deletion of neuronal NOS (nNOS; Ref. 13) have been used to elucidate the role of NO in lower urinary tract function and dysfunction (7, 33), but these studies have provided conflicting results on the involvement of NO in normal micturition. Some residual NOS activity is known to persist in nNOS knockout mice (13).

It is now widely recognized that NO activates soluble guanylate cyclase and increases tissue levels of cGMP (25). Nerve-induced relaxation of the urethra in the rabbit and sheep is associated with an increase in the smooth muscle content of cGMP but not cAMP (10, 26). Inhibition of NOS by Nω-nitro-L-arginine (L-NNA) prevented both the increase in cGMP content and the urethral relaxation. Furthermore, in the presence of the cGMP phosphodiesterase inhibitor zaprinast the increase in cGMP levels was enhanced (26). Thus cGMP seems to have a second messenger role in the urethra, and the NO-guanylate cyclase-cGMP system is activated during NANC nerve-mediated relaxation. For bladder relaxation, cGMP formation seems to be more important than the NO-cGMP pathway (24, 26, 35).

The second messenger cGMP regulates three main classes of effector proteins: 1) cGMP-dependent protein kinases, 2) cGMP-gated ion channels, and 3) phosphodiesterases (32), and under certain conditions also cAMP-dependent kinases (20). NO-mediated smooth muscle relaxation is proposed to be induced by cGMP through activation of cGMP-dependent protein kinases (18). Two different forms of cGMP-dependent protein kinase type I (cGKI) and type II (cGKI) have been demonstrated, but only cGKI is expressed in smooth muscle (36). The importance of cGKI for smooth muscle function was tested recently in cGKI-null mice (29). Inactivation of the cGKI gene in mice abolished NO-cGMP-dependent relaxations of smooth muscle and caused vascular and intestinal dysfunctions (29), including hypertension, intestinal distension, and gastric fundus hypertrophy.

Because NO-mediated urethral smooth muscle relaxation is proposed to be induced by cGMP, mice lacking the cGKI enzyme (29) can provide important information on the role of NO in the lower urinary tract function and dysfunction (7, 33), but these studies have provided conflicting results on the involvement of NO in normal micturition. Some residual NOS activity is known to persist in nNOS knockout mice (13).
tract. In the present study, we assessed in vitro and in vivo the functional characteristics of lower urinary tract smooth muscle from mice lacking cGKI expression. A preliminary report has been published previously (28).

METHODS

Animals

The generation of cGKI-null mice has been described in detail previously (29). Fifteen cGKI−/− and 15 litter-matched cGKI+/+ mice (22 females and 8 males) were used for in vitro studies. Female cGKI−/− (n = 4) and cGKI+/+ (n = 4) were used for cystometric investigations. As previously described (29) the cGKI−/− mice have higher blood pressure (134 ± 2 mmHg) than wild-type mice (118 ± 2 mmHg). The experimental procedures were approved by the Animal Ethics Committee of Lund University.

In Vitro

Recording of mechanical activity. The mice were killed by CO2 asphyxia, and the bladder and urethra were removed en bloc. The bladder and urethra were separated at the level of the bladder neck, and semicircular strips (1 × 2 × 5 mm) were prepared from the bladder. One longitudinal strip (1 × 2 × 5 mm) was prepared from the proximal and middle part of the female urethra. Urethral tissue from male mice was not used for functional studies because of the possible interference of prosthetic tissue. The muscle strips were transferred to thermostatically controlled (37°C) 5-ml tissue baths containing Krebs solution aerated with 5% CO2-95% O2. The strips were attached to two hooks by silk ligatures. One hook was attached to a force transducer FT03C (Grass Instrument, Quincy, MA) and the other to a movable unit that allowed adjustment of passive tension. Isometric tension was recorded using a Grass polygraph (model 7D). Transmural stimulation of nerves was accomplished by means of two platinum electrodes placed on either side of the preparations. Stimulation of nerves was performed with a Grass S48 stimulator delivering single square-wave pulses (duration 0.5 ms) at the voltage giving maximal response. The train duration was 5 s, the stimulation interval was 120 s, and the polarity of the electrodes was shifted after each pulse by means of a polarity changing unit.

Experimental procedures. During an equilibration period of 45–60 min the preparations were stretched to a stable passive tension of 4 mN (bladder) and 2 mN (urethra). After this period, each experiment was started by exposing the preparations to a K+ (124 mM) Krebs solution. In bladder preparations, frequency-response relationships (2–50 Hz) to electrical field stimulation (EFS) and concentration-response relationships to carbachol (10 nM-1 mM) were recorded. The EFS-induced contractile response was also characterized by addition of scopolamine (1 μM). Relaxant responses to 8-bromo-cGMP (8-BrcGMP; 0.1 μM-0.1 mM) and forskolin (1 nM-1 μM) were recorded in preparations precontracted by carbachol (10 μM). Urethral preparations were pretreated with guanethidine (1 μM) and scopolamine (1 μM) for 30 min. The relaxant responses to EFS (2–32 Hz; in the absence or presence of t-NAME, 0.1 mM) and to SIN-1 (10 nM-0.1 mM), 8-BrcGMP (10 nM-0.1 mM) and forskolin (1 nM-1 μM) were studied in urethral preparations precontracted by arginine vasopressin (AVP, 0.1 μM). Bladder and urethral preparations from cGKI−/− and cGKI+/+ mice were examined in parallel, and each preparation was subjected to two treatments. Concentration-response relationships were determined by cumulative addition of increasing drug concentrations. A new concentration was added when the effect of the preceding one had reached a plateau.

Immunohistochemistry. The tissues were immersion fixed for 4 h in cold 4% formaldehyde in 0.1 M PBS and then rinsed in PBS containing 15% sucrose for 2–3 days. Both fixation and rinsing were performed at 4°C, after which the specimens were frozen in isopentane at −40°C and stored at −70°C before sectioning. Tissue sections were cut at a thickness of 10 μm and preincubated with PBS containing 0.25% Triton X-100 for 2 h at room temperature. Incubation with primary antisera was performed overnight in the presence of rabbit antisera raised against cGKI (1:1,000; Ref. 29) or sheep antisera raised against NOS (1:4,000; Dr. P. C. Emson, Babraham Institute, Cambridge, UK). The primary antisera were diluted in PBS containing 1% BSA and 0.25% Triton X-100. For the visualization of the immunoreactive products, the sections were rinsed in PBS and then incubated for 90 min with either FITC-conjugated donkey anti-sheep IgG (1:80; Sigma Chemical) or Texas Red-conjugated affinity purified F(ab)’2 fragments of donkey anti-rabbit IgG (1:160; Jackson Immunoresearch Laboratories, West Grove, PA), diluted in PBS containing 1% BSA. All incubations with primary and secondary antisera were performed at room temperature in moisture chambers.

For double-label immunofluorescence of NOS and cGKI, sections were first incubated with one primary antiserum overnight, rinsed in PBS, and then incubated with the other antiserum. After being rinsed, the sections were incubated for 90 min with FITC-conjugated donkey anti-sheep IgG, rinsed, and then incubated for 90 min with Texas Red (TR) conjugated donkey anti-rabbit IgG. The sections were finally rinsed and mounted in PBS-glycerol with p-phenylenediamine to prevent fluorescence fading. An Olympus epifluorescence microscope equipped with appropriate filter settings for TR- and FITC-immunofluorescence was used. Because cross-reactions with antigens sharing similar sequences cannot be excluded, the structures demonstrated are referred to as, e.g., NOS immunoreactive.

In Vivo

Surgical procedures. Mice were anesthetized with ketamine (Ketalar, Parke Davis, Barcelona, Spain; 75 mg/kg im) and xylazine (Rompun, Bayer, Leverkusen, Germany; 15 mg/kg im). The abdomen was opened by a lower midline incision, and the bladder and urethra were identified. A polyethylene catheter (Portex, Kent, UK) was implanted into the bladder dome as previously described in rats (22). The catheter was tunneled subcutaneously, and an orifice was made at the back of the animal.

Cystometric investigations. Cystometric investigations were performed without any anesthesia 3 days after bladder catheter implantation. The conscious mice were placed without any restrain in a mouse metabolic cage (Gazzada, Buguggiat, Italy), which enabled measurement of micturition volumes by means of a fluid collector connected to a force displacement transducer (FT03D; Grass Instrument). The bladder catheter was connected via a T-tube to a pressure transducer (P23 DC; Statham Instruments, Oxnard, CA) and a microinjection pump (CMA 100, Carnegie Medicine, Solna, Sweden). Room temperature saline was infused into the bladder at a rate of 1.5 ml/h. The rate of infusion of saline was chosen on the basis of our own pilot experiments. Intravesical pressure and micturition volumes were recorded continuously on a Grass polygraph (model 7E, Grass Instrument).
At the beginning of the cystometry, the bladder was emptied through the bladder catheter. Because each micturition comprised not more than one or two droplets, the inner collecting funnel of the metabolic cage was coated with a fine silicon spray to minimize volume loss. A stabilizing period of 60–80 min was needed for the voiding pattern to get stabilized; thereafter a 30-min period with reproducible micturition cycles was used for evaluation. The following cystometric parameters were investigated: micturition pressure (the maximal bladder pressure during micturition), basal pressure (the lowest bladder pressure during filling), threshold pressure (bladder pressure immediately before micturition), bladder capacity (residual volume at the latest previous micturition plus volume of the infused saline at the micturition), micturition volume (volume of the expelled urine), residual urine (bladder capacity minus micturition volume), and intercontraction intervals (intervals between micturition contractions). The occurrence of spontaneous changes in intravesical pressure (nonvoiding contractions) was noted.

Drugs and solutions. The Krebs solution used had the following composition (in mM): 119 NaCl, 4.6 KCl, 1.5 CaCl₂, 1.2 MgCl₂, 15 NaHCO₃, 1.2 NaH₂PO₄, and 5 glucose. The following drugs were used in functional in vitro studies: scopolamine hydrochloride, L-NNA, carbachol, guanethidine, forskolin, 8-BrcGMP (Sigma Chemical, St Louis, MO), AVP acetate (Peninsula Laboratories, Belmont, CA). SIN-1 was prepared and then stored at −80°C. K⁺ Krebs solution (124 mM) was prepared by replacing NaCl with equimolar amounts of KCl.

Analysis of data. The results are given as means ± SE. In in vitro studies, an ANOVA for repeated measurements followed by Fisher’s post hoc test was used to compare the genotypes, and Student’s unpaired t-test was used to compare the response at separate concentrations or frequencies. In in vivo studies Student’s unpaired t-test was used for comparisons between the genotypes. A probability level of <5% was accepted as significant.

RESULTS

There was no difference in body weight between the genotypes on the day of the experiment (cGKI+/+ 21 ± 0.8 g; cGKI−/− 19 ± 1.3 g; n = 13 for each group). The ratio of bladder wt (mg) to body wt (g) were 1.2 ± 0.08 (cGKI+/+) and 1.4 ± 0.09 (cGKI−/−). The difference was not significant.

Immunohistochemistry

In wild-type mice, an intense cGKI immunoreactivity was found in the urethral smooth muscle layer and within the lamina propria (Fig. 1A). In the bladder, the smooth muscle was immunoreactive for cGKI, but the intensity was markedly weaker than in the urethra (Fig. 1C). Moreover, the smooth muscle of intramural vessels showed distinct labeling for cGKI (Fig. 1A and C). cGKI immunoreactivity was also revealed in neuronal structures (Fig. 1E). In cGKI-deficient mice, all smooth muscle and neuronal structures were devoid of immunoreactivity to cGKI (Fig. 2, A and C).

In the urethral smooth muscle and in the lamina propria, NOS-immunoreactive nerve fibers were abundant (Fig. 1B). In the bladder, NOS-immunoreactive cell bodies and coarse nerve trunks were found at the serosal side, whereas thin nerve fibers were distributed in the smooth muscle layer (Fig. 1D). cGKI immunoreactive structures and NOS-immunoreactive nerves were found close to each other but were never found to overlap within the same cells. Notably, in same nerve trunks, cGKI-positive fibers were found to intermingle with NOS-positive fibers (Fig. 1, E and F). The supply and distribution of NOS-immunoreactive nerves appeared to be unaltered in cGKI−/− mice (Fig. 2, B and D).

Functional Responses of the Urethra

In urethral preparations of cGKI+/+ mice, EFS (2–32 Hz) elicited frequency-dependent relaxations (Figs. 3 and 4). The maximum relaxation at 32 Hz amounted to 32 ± 6% (n = 7) of the AVP-induced tension. The relaxations were abolished by L-NNA (0.1 mM) and instead a contractile response to stimulation was generally found (Figs. 3 and 4). Frequency-dependent relaxations were significantly (ANOVA, P < 0.01) impaired in cGKI-deficient mice. The response to EFS was practically abolished (8 ± 3%, n = 6, at 32 Hz) in urethral strips from cGKI−/− mice, although a small relaxation generally appeared at 16–32 Hz (Figs. 3 and 4). This relaxant response was not inhibited by L-NNA (Fig. 4). The relaxant responses to the NO donor SIN-1 and the cGMP analog 8-BrcGMP were also investigated. In wild-type mice SIN-1 and 8-BrcGMP induced relaxations that amounted to 49 ± 7% (n = 6) and 60 ± 10% (n = 5), respectively (Fig. 5). The responses induced by SIN-1 and 8-BrcGMP were significantly (ANOVA P < 0.01) impaired in cGKI-deficient animals (Fig. 5). In addition, the relaxant response evoked by the adenylate cyclase activator forskolin was significantly (ANOVA, P < 0.001) attenuated in cGKI−/− mice (Fig. 6). In cGKI+/+ mice, a maximal relaxation of 73 ± 8% (n = 5) was found in response to forskolin (1 μM). Addition of 1 μM forskolin to cGKI-deficient urethral strips produced an average relaxation of 14 ± 4% (n = 5) (Fig. 6).

Functional Responses of the Bladder

The bladder weight of cGKI-deficient mice was not different from the bladder weight of wild-type mice (cGKI+/+ 26 ± 1.9 mg, cGKI−/− 25 ± 2.1 mg, n = 12). There were no differences in K⁺-induced bladder contractions between the genotypes (cGKI+/+ 11 ± 0.9 mN, n = 9; cGKI−/− 10 ± 1.4 mN, n = 8). Carbachol (0.1 μM-30 μM) and EFS (2–50 Hz) evoked contractions of the bladder strips. The contractile response to carbachol and EFS did not differ in cGKI−/− compared with cGKI+/+ mice (Fig. 7, A and B). The noncholinergic bladder contractions that appeared after scopolamine treatment were also similar (Fig. 7B). The relaxant response to 8-BrcGMP was more pronounced (ANOVA, P < 0.001) in bladder strips from wild-type mice than in bladder strips from cGKI-deficient mice (Fig. 8). However, forskolin-induced bladder relaxations were of the same amplitude in wild-type (34 ± 2%, n = 4) and cGKI−/− mice (32 ± 6%, n = 6).
**Cystometric Findings**

Continuous cystometry in four female cGKI−/− mice and four female wild-type controls revealed distinct differences between the genotypes. The micturition pattern in cGKI+/+ mice was regular (Fig. 9A) and nonvoiding contractions in between micturitions were rarely seen. In contrast, the micturition pattern in cGKI-deficient mice was irregular (Fig. 9B) and characterized by frequent voidings and nonvoiding bladder contractions. In cGKI−/− mice, the intercontraction intervals were significantly (P < 0.001) shorter (2.7 ± 0.23 min, range 2.03–3.43) compared with controls (5.8 ± 0.31 min, range 4.70–6.60). Micturition pressure, bladder capacity, and micturition volume were reduced in cGKI−/− mice (Table 1), although these changes did not reach statistical significance.

**DISCUSSION**

If the activity of the NO-cGMP pathway in the lower urinary tract is reduced, the ability of the bladder outlet to relax may be impaired and micturition disorders develop. Detrusor contractions against a partly closed urethra can result in incomplete emptying of the bladder, and bladder overactivity may be due to lack of coordination between the bladder and its outlet. The present study, using genetic disruption of a target protein (cGKI) for NO-mediated responses (29), represents a new strat-
egy for studies of the importance of the NO-cGMP pathway for lower urinary tract function. Our results showed that urethral smooth muscle relaxations in response to nerve stimulation and agents acting through the NO-guanylate cyclase-cGMP pathway are abolished or markedly reduced in cGKI−/− mice. These results suggest that cGKI is the main effector protein for NO-cGMP-induced relaxations in the mouse urethra and that other known targets for cGMP such as cation channels, phosphodiesterases, and cAMP-dependent kinases are of minor importance. Immunohistochemical studies showed that the distribution of NOS-containing nerves in the smooth muscle of cGKI-deficient mice was similar as in controls, confirming that the source of NO production was unaltered. Moreover, the integrity of the cGMP pathway proximal to the kinase is not affected in cGKI-deficient mice (29). At higher frequencies of stimulation, a small urethral relaxation persisted in cGKI−/− mice that was unaffected by NOS inhibition. Similar, non-NO-mediated relaxations have been reported in urethral tissue from other species.

Fig. 2. Double immunolabeling of cGKI (A, C) and NOS (B, D) in the same section of the urethra (A, B) and bladder (C, D) of cGKI−/− mice. Immunoreactivity to NOS but not to cGKI is demonstrated. Scale bar, 50 μm.

Fig. 3. Original tracings showing the responses to electrical field stimulation (EFS; 0.5-ms pulses, frequency 2–32 Hz) and to SIN-1 in urethral preparations from cGKI+/+ and cGKI−/− mice. L-NAME converts the relaxation in preparations from cGKI+/+ mice into a contraction.
but the identity of the transmitter(s) is unknown.

Investigations of the lower urinary tract function and morphology of nNOS knockout mice have so far been inconclusive (7, 33). Studies in male nNOS-deficient mice revealed markedly dilated bladders with muscular hypertrophy, findings compatible with deficient urethral relaxation and increased outflow resistance (7). In addition, voiding studies revealed an increased micturition frequency in male nNOS-deficient mice. In contrast to the findings in male nNOS knockouts, similar investigations in female nNOS-deficient mice, revealed no marked differences in voiding function between nNOS-deficient mice and controls (33). It was speculated that nNOS-deficient mice had developed alternative pathways to compensate for the lack of nNOS. The reason lower urinary tract function in male mice should be more sensitive to NO depletion than in female mice is unclear. The difference is surprising because at least in rats it has been suggested previously that female individuals depend more on NO for urethral relaxation than males (9). In our study using both female and male mice, there was no sex difference noted in the in vitro functional or morphological response to targeted disruption of the cGKI gene. No macroscopical signs of outflow obstruction or bladder hypertrophy were seen in either male or female cGKI−/− mice, and the bladder weight of cGKI−/− mice was not different from the bladder weight of wild-type animals, which is in line with findings in female nNOS-deficient mice (33).

A novel isoform of nNOS, different from cerebellar NOS in containing an additional 34 amino acids, has been reported recently (16, 21). This isoform of NOS is particularly well expressed in urogenital tissue. Moreover, the nNOS-μ isoform is likely to account for the residual NOS activity that persists in...
nNOS knockout mice (13). With the residual NOS activity in mind, results obtained on lower urinary tract function in nNOS knockout mice should be interpreted with some caution.

In contrast to the situation in the bladder, the cGKI−/− mice did develop gastric fundus hypertrophy (29), but whether this means that the sphincters in the gastrointestinal tract depend more on the NO-cGMP pathway than the urethra is not known. However, in addition to NO-mediated inhibition, other possible mechanisms for mediating the decrease in outflow resistance at micturition exist. First, parasympathetic inhibition of the excitatory sympathetic tone by presynaptic interactions is thought to be one mechanism, and second, a passive urethral relaxation generated by the contracting detrusor has been discussed (1). It is possible that the presence of these latter mechanisms enable cGKI-deficient mice to maintain urethral relaxation in vivo.

Cystometric studies on awake cGKI−/− mice revealed alterations in bladder function suggesting bladder overactivity. There was a highly significant decrease in intercontraction intervals, and the nonvoiding bladder contractions found in the cGKI−/− mice suggest that the ability of the bladder to store urine at low baseline pressure is impaired. NO can be released from urothelial cells and sensory nerves (5), and it has been suggested that NO-cGMP depresses the excitability of bladder afferent by modulating neuronal Ca2+ currents (38). Consequently, a lack of inhibitory NO or depletion of target proteins for NO-cGMP could possibly increase afferent activity and result in bladder overactivity. Whether the proposed regulatory effect of NO on bladder afferent excitability involves cGKI is not known, but cGKI expression has been demonstrated in sensory neurons such as rat dorsal root ganglion (30).

It has been reported previously that nerve fibers in the lower urinary tract express cGMP (31), which is supported by findings in the present study showing neuronal cGKI immunoreactivity. cGKI-positive nerve fibers were often found in the vicinity of, but were never overlapping, NOS immunoreactive fibers or nerve cell bodies. NO has been reported to modulate neurotransmission in several tissues such as the guinea pig ileum (12) and the mouse bladder (19). The modulatory effect of NO on neurotransmission in the guinea pig ileum (12) and in the mouse bladder (unpublished observations) could be inhibited by guanylate cyclase inhibitors, suggesting involvement of cGMP. Thus although NO has the potential to modify neurotransmitter release via the cGMP-cGKI pathway, nerve-evoked contractions of bladder strips from cGKI−/− mice were not different from controls, suggesting that the endogenous NO-cGMP-cGKI pathway is of minor importance for regulation of normal bladder contractions.

Although some studies have proposed a role for NO in detrusor muscle relaxation during filling (8, 34), compared with the outflow region the detrusor has a low sensitivity to NO (2). There were no functional differences in K+-, carbachol-, or EFS-induced bladder contractions.

Fig. 7. Contractile responses to carbachol (A) and EFS (B) in bladder preparations from cGKI+/+ (open symbols) and cGKI−/− (solid symbols) mice. EFS was performed before (circles) and after treatment with scopolamine (triangles). Data are expressed as means ± SE (n = 6 or 8).

Fig. 8. Relaxant response to 8-Br-cGMP in bladder preparations from cGKI+/+ (open circles) and cGKI−/− (solid circles) mice. Data are expressed as means ± SE (n = 6). **P < 0.01, ***P < 0.001, Student’s t-test.
contractility in vitro between the cGKI genotypes. However, the relaxant response to 8-BrcGMP was, as expected, more pronounced in bladder strips from wild-type mice than in bladder strips from cGKI−/− mice. A pronounced immunoreactivity to cGKI was found in the smooth muscle of intramural vessels in bladders from wild-type mice, but not from cGKI−/− mice. The bladder wall blood flow undergoes cyclic changes with filling and emptying (3), and these changes seem to be related primarily to the NO pathway (15). Thus it cannot be excluded that an impaired microcirculation of the bladder wall contributes to the found bladder overactivity in cGKI-deficient mice.

Previous studies have proposed that cAMP may relax smooth muscle by cross-activation of cGKI (14, 17). However, when investigated in cGKI−/− mice, the cAMP-induced relaxations of vascular and intestinal smooth muscle were found to be independent on cGKI (29). In our study, relaxations evoked by the adenylate cyclase-cAMP activator forskolin were found to be impaired in the urethra but not in the bladder of cGKI−/− mice. However, whether relaxations induced with forskolin are mediated exclusively by cAMP remains unclear because forskolin, at least in the sheep urethra (10), was found to cause a threefold increase in tissue levels of cGMP. Thus the results in the present study do not necessarily suggest a cross-talk between cAMP and cGKI in the mouse urethra.

In conclusion, our data showed that inactivation of the cGKI gene abolished NO-cGMP-dependent relaxations of urethral smooth muscle and resulted in hyperactive voiding. It is suggested that certain voiding disturbances may be associated with impaired NO-cGKI signaling.

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Table 1. Cystometric parameters recorded from cGKI+/+ and cGKI−/− mice

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<tr>
<td>MP</td>
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<td>ICI</td>
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Values are means ± SE; n = 4 mice/group. MP, micturition pressure (cmH2O); TP, threshold pressure (cmH2O); BP, basal pressure (cmH2O); BC, bladder capacity (ml); MV, micturition volume (ml); RV, residual volume (ml); ICI, intercontraction intervals (min). *P < 0.001, Student's t-test.
REFERENCES


