Presence of Phosphodiesterase Type 5 in the Spinal Cord and its Involvement in Bladder Outflow Obstruction Related Bladder Overactivity

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Abbreviations and Acronyms

BC = bladder catheterization
BCap = bladder capacity
BO = bladder overactivity
BP = basal bladder pressure
cGMP = cyclic guanosine monophosphate
Ct = threshold cycle
GAPDH = glyceraldehyde-3-phosphate dehydrogenase
IC = intrathecal (subarachnoid) catheterization
IMI = intermicturition interval
IMP = intermicturition pressure
L-NAME = N-nitro-L-arginine methyl ester
LUTS = lower urinary tract symptoms
MMP = maximum micturition pressure
MV = micturition volume
NO = nitric oxide
PCR = polymerase chain reaction
PDE5 = phosphodiesterase type 5
PUO = partial urethral obstruction
RV = post-void residual volume
SA = spontaneous bladder activity
TP = threshold pressure

Purpose: Phosphodiesterase type 5 inhibitors were recently introduced as a new treatment option for men with lower urinary tract symptoms. Safety and clinical effectiveness are well documented but the mode of action is still unclear. We determined and compared the expression of phosphodiesterase type 5 in the spinal cord of normal (sham operated) rats and rats with partial urethral obstruction induced bladder overactivity. We also assessed the urodynamic effects of intravenously and intrathecally administered sildenafil in the rats to determine whether phosphodiesterase type 5 inhibitors exert effects on the sacral spinal cord.

Materials and Methods: A total of 65 male Sprague-Dawley® rats were used for molecular/morphological and functional experiments. Bladder overactivity was induced via surgical partial urethral obstruction in 39 of 65 rats. Spinal phosphodiesterase type 5 expression was assessed by histology and polymerase chain reaction. The effects of sildenafil administered intravenously or intrathecally were studied urodynamically.

Results: Phosphodiesterase type 5 was expressed in various regions of the lumbosacral spinal cord, including the sacral regions of micturition control. Expression was similar in normal rats and rats with partial urethral obstruction/bladder overactivity. In normal rats intravenous and intrathecal sildenafil had no urodynamic effect. When administered intravenously and intrathecally to rats with partial urethral obstruction/bladder overactivity, sildenafil decreased micturition frequency and bladder pressure. Doses tested intrathecally had no effect when given intravenously.

Conclusions: Phosphodiesterase type 5 is expressed in the rat spinal cord. Intravenous sildenafil may exert part of its urodynamic effect in rats with partial urethral obstruction/bladder overactivity via an effect on the sacral spinal cord.

Key Words: urinary bladder; sildenafil; injections, spinal; administration, intravenous; lower urinary tract symptoms

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See Editorial on page 1161.

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PHOSPHODIESTERASE type 5 inhibitors are first line therapy for men with erectile dysfunction.[1] The drugs are also currently used to treat male LUTS. Large double-blind, randomized, placebo controlled trials showed that all well established PDE5 inhibitors, including sildenafil, vardenafil and tadalafil, are safe and effective in men with LUTS associated with benign prostatic hyperplasia regardless of whether they have concomitant erectile dysfunction.[2–4] However, while the clinical safety and efficacy of the drugs are well documented, the mechanisms behind the beneficial effects on LUTS have not been established.[5]

PDE5s are expressed in the prostate, urethra, bladder and lower urinary tract vasculature.[5] Reports show that PDE5 inhibitors relax prostatic tissue,[6] decrease urethral pressure,[7] relax the detrusor muscle[8] and increase bladder oxygenation.[9] However, there remains doubt which, if any, observed effects are responsible for the clinically relevant actions of the drugs.

We determined the presence of PDE5 in the spinal cord via immunohistochemistry and PCR, and compared PDE5 expression in the spinal cord regions of micturition control in rats with normal bladder function and those with PUO induced BO. We also evaluated the urodynamic effects of intrathecal (subarachnoid) and intravenous sildenafil in normal and PUO/BO rats to determine whether PDE5 inhibitors act on the sacral spinal cord.

MATERIALS AND METHODS

A total of 65 male Sprague-Dawley rats weighing 250 gm were housed at the Walter Brendel Centre of Experimental Medicine, Ludwig-Maximilians-University, Munich, Germany. Experiments were approved by the local animal care and use committee.

Of the 25 rats used for PCR and immunostaining experiments 14 underwent PUO. After PUO 3 rats were sacrificed due to acute urinary retention, leaving 6 with and 6 without obstruction for PCR, and 5 with and 5 without obstruction for immunostaining experiments.

For functional experiments we used 40 rats, of which 25 underwent PUO. The mortality rate was 24%. After IC 9 rats were sacrificed due to persistent paralysis. On final functional analysis 13 rats served as normal controls, in which sildenafil was given intravenously in 7 and intrathecally in 6. In the 12 rats in the PUO group sildenafil was tested intravenously in 6 and intrathecally in 6.

Partial Urethral Obstruction

PUO was created as described by Melman et al.[10] Briefly, the urethra was accessed via a perineal incision and isolated from the corpora cavernosa. The urethra was then partially ligated with a 3-zero polypropylene suture using a 0.4 mm (26 gauge) metal rod as a temporary placeholder. After 14 days, rats that underwent PUO were used for molecular/morphological or functional experiments.

Quantitative PCR

Rats were decapitated and the spinal cord was removed by hydraulic ejection.[11] Lumbar thickening was used as a landmark to identify sacral spinal cord segments,[12] which were immediately snap frozen in liquid nitrogen. RNA from frozen tissues was isolated using the RNeasy® Mini Kit. For isolation 30 mg tissue were homogenized using the FastPrep®-24 system with matrix A (MP™ Biomedicals). RNA concentrations were measured spectrophotometrically. Reverse transcription to cDNA was performed with 2 μg isolated RNA using the Reverse Transcription System (Promega®). Reverse transcriptase-PCR for PDE5 and the housekeeping gene GAPDH was performed with a LightCycler® using primers provided by SA Biosciences™ as ready-to-use mixes based on the RefSeq Accession No. NM_133584 for rat PDE5 and NM_017008 for rat Gapdh (http://www.ncbi.nlm.nih.gov/projects/RefSeq/key.html). PCR reactions were performed in a volume of 25 μl containing 8 μl LightCycler FastStart DNA Master^{PLUS} SYBR Green I, 2 μl template, 2 μl primer and 13 μl water. Denaturation was performed for 10 minutes at 95C. Amplification was done for 45 cycles for 15 seconds at 95C, followed by 60 seconds at 60C. Primer and amplification specificity was demonstrated by subsequent analysis of melting points, which revealed single peaks for each target. Results are expressed as the number of cycles at which the fluorescence signal exceeded a defined threshold.

Immunostaining

Spinal cord (L6-S2) tissue[13] was harvested after intracardial perfusion with ice-cold heparinized saline for 10 minutes, followed by 4% paraformaldehyde for 20 minutes.[14] Tissue was post-fixed for 3 days in 4% paraformaldehyde, embedded in paraffin and cut into transverse sections according to standard protocols. Immunohistochemistry for PDE5 was performed on a Ventana® Benchmark staining device after deparaffinization using rabbit polyclonal antibody (ab14672, Abcam®)[15] diluted 1:200 in antibody diluent (Ventana). Pretreatment was done using a standard program (CC1, Ventana). For detection we used the iView™ DAB Detection Kit.

Functional Experiments

BC and IC. BC and IC were performed as described previously.[16,17] IC was done immediately after BC and during the same anesthesia. For IC the rat was mounted in a stereotactic frame. A small nuchal midline incision was made rostral of the BC exit. The atlanto-occipital membrane was punctured and a 32 gauge intrathecal catheter (CS-1, ReCathCo, Allison Park, Pennsylvania) was inserted and pushed forward to the sacral level. Correct positioning of intrathecal catheters at L6-S2[13] was confirmed by methylene blue injection at necropsy after the experiments.

Cystometry (urodynamics). Cystometry without anesthesia was performed 3 days after BC, as previously described in detail.[16] The cystometric parameters investigated were IMI in minutes, BCap (infusion rate per minute × IMI) in ml, MV in ml, RV (BCap − MV) in ml, BP (minimum BP between 2 micturitions) in cm H_2O, IMP (mean BP between 2 micturitions) in cm H_2O, TP (BP at micturition contraction curve per peak onset)

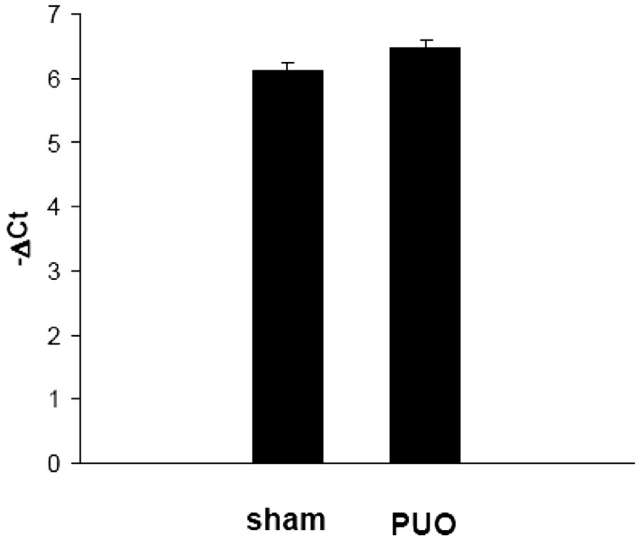

Figure 1. Mean ± SEM PDE5 mRNA expression in spinal cord of 6 sham operated and 6 PUO rats, as detected by quantitative reverse transcriptase-PCR. PDE5 values were normalized to housekeeping gene GAPDH and are expressed as −ΔCt.

in cm H_2O, MMP in cm H_2O and SA (IMP − BP) in cm H_2O.

Drug administration. Stock solution of sildenafil citrate (100 mM) (Tocris Bioscience, Ellisville, Missouri) was made in dimethyl sulfoxide and stored at −80C. Subsequent dilutions were made in saline on the day of the experiment. After 45 minutes of ongoing cystometry, sildenafil was administered intrathecally (1 μg) as a bolus (volume 10 μl) slowly injected during 1 minute or intravenously (1 μg) as a bolus (0.1 ml) in the rat tail vein. Cystometry continued another 45 minutes. Rats that received sildenafil intravenously then received the next dose (3 mg/kg) and cystometry continued another 45 minutes. Sildenafil doses were chosen based on published information.[18]

Statistical Analysis

Results are shown as the mean ± SEM. When normally distributed, we used the Student t test between groups or the Student paired t test within groups (before/after drug) for comparison. When more than 1 treatment was administered, as in the intravenous sildenafil groups, we used 1-way repeated measures ANOVA, followed by the Student-Newman-Keuls test. SigmaPlot™ 11.0 was used for statistical analysis with $p < 0.05$ considered statistically significant.

RESULTS

Polymerase Chain Reaction

PDE5 mRNA was detectable in spinal cord tissue from sham operated and PUO rats. Average −ΔCt was similar in the 2 groups (6.13 ± 0.14 and 6.47 ± 0.17, respectively), indicating similar PDE5 mRNA expression (fig. 1).

Immunostaining

Immunohistochemistry for PDE5 in the lumbosacral spinal cord showed diffuse staining of the gray matter without any obvious laminar pattern, while no staining was observed in the white matter. In addition to diffuse faint staining of the neuropil, more intense diffuse cytoplasmic staining of neuronal cell bodies was observed in the ventral or dorsal horn (fig. 2). No difference in localization or density was observed between the experimental groups.

Functional Data

Urethral obstruction for 14 days decreased IMI by 54%, BCap by 52% and MV by 54% (each $p < 0.05$). There was no effect on RV. We noted increases in BP (195%), IMP (199%), TP (181%) and MMP (77%) (each $p < 0.05$). SA increased 231% ($p < 0.05$, see table). Body weight did not differ between obstructed and healthy rats (mean 255 ± 11 vs 242 ± 8 gm). Bladder weight in obstructed rats was significantly greater than in nonobstructed rats (mean 758 ± 104 vs 247 ± 12 mg, $p < 0.05$).

Intravenous sildenafil had no urodynamic effect in healthy rats at the dose of 1 μg or 3 mg/kg body

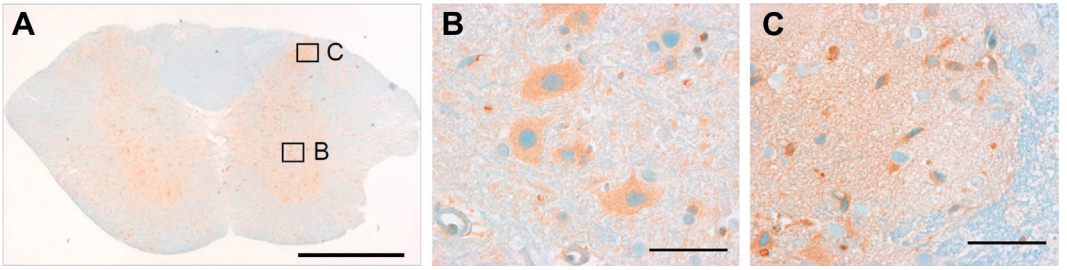

Figure 2. PDE5A immunohistochemistry. Transverse section of lumbosacral rat spinal cord reveals diffuse gray matter staining (*A*). Insets, higher magnification (*B* and *C*). Large motoneurons in ventral horn showing diffuse cytoplasmic staining of moderate intensity with faint neuropil staining (*B*). In dorsal horn faint diffuse staining of neuropil and more intense cytoplasmic staining of several cell bodies can be seen (*C*). Scale bars indicate 1 mm (*A*) and 50 μm (*B* and *C*).

weight (about 750 μg sildenafil in a rat weighing 250 gm), while intrathecal sildenafil (1 μg) had no urodynamic effect in normal rats (see table).

In obstructed rats intravenous sildenafil at a dose of 1 μg had no urodynamic effect (see table). However, at a dose of 3 mg/kg body weight intravenous sildenafil increased IMI by 76%, BCap by 69% and MV by 76% (each $p < 0.05$). There was no effect on RV. We noted decreases in BP (43%), IMP (36%), TP (37%) and MMP (35%) (each $p < 0.05$). SA decreased 40% ($p < 0.05$, see table).

Intrathecal sildenafil (1 μg) in obstructed rats increased IMI by 58%, BCap by 61% and MV by 39% (each $p < 0.05$). There was no effect on RV. We noted decreases in BP (45%), IMP (59%), TP (50%) and MMP (30%) (each $p < 0.05$). SA decreased 75% ($p < 0.05$, see table).

Vehicle administered intravenously or intrathecally had no effect on any cystometric parameter in any group. There was also no time effect in our pilot experiments (data not shown).

DISCUSSION

To our knowledge this study is the first to show the direct presence of PDE5 in the lumbosacral spinal cord. PDE5 was expressed in regions relevant for afferent micturition control (dorsal horn layers). However, PDE5 was also expressed in other areas of the sacral spinal cord, such as the motoneurons of the ventral horn, suggesting that spinal PDE5 is relevant for micturition control as well as for various other functions. PDE5 is present in cultured rat lumbar spinal motoneurons and, based on the protective effects of PDE5 inhibition against neurotoxic stimuli, a potential role for PDE5 inhibitors was suggested in certain motoneuron diseases.[19] In a diabetic neuropathic mouse model sildenafil improved sensory and motoneuron conducting velocities.

Our morphological findings were confirmed by PCR experiments, which revealed spinal expression of PDE5 RNA. The presence of PDE5 RNA in spinal cord tissues was previously reported by others.[20]

We also assessed and compared spinal PDE5 expression in normal and PUO/BO rats. No difference was found between the groups histologically or in PCR experiments. This finding was surprising because intrathecal sildenafil had no urodynamic effect in normal rats but pronounced effects in obstructed rats. However, the presence of PDE5 alone might not reflect its activity since it could also be controlled allosterically.[21] In addition, PUO induced changes in afferent neurons,[22,23] primarily those residing in the dorsal root ganglia. This might affect their sensitivity to cGMP and, thus, to PDE5 inhibitors.

Sildenafil given intravenously had no urodynamic effect in normal rats but intravenous

Urodynamic parameters in nonobstructed and obstructed rats

Sildenafil Administration (No. rats)	Mean ± SEM IMI (mins)	Mean ± SEM BC (ml)	Mean ± SEM MV (ml)	Mean ± SEM RV (ml)	Mean ± SEM BP (cm H_2O)	Mean ± SEM IMP (cm H_2O)	Mean ± SEM TP (cm H_2O)	Mean ± SEM MMP (cm H_2O)	Mean ± SEM SA (cm H_2O)
Before drug:									
Nonobstructed (13)	5.27 ± 0.25	0.88 ± 0.04	0.89 ± 0.06	0.00 ± 0.01	5.5 ± 0.8	9.1 ± 1.1	16.5 ± 0.9	59.6 ± 4.5	3.5 ± 0.7
Obstructed (12)	2.45 ± 0.27*	0.42 ± 0.04*	0.41 ± 0.05*	0.03 ± 0.01	16.2 ± 1.4*	27.2 ± 2.2*	46.4 ± 3.2*	105.3 ± 7.8*	11.6 ± 1.4*
				Nonobstructed					
Intravenous (7):									
Before drug	5.71 ± 0.36	0.95 ± 0.06	0.96 ± 0.09	0.02 ± 0.01	5.7 ± 1.0	9.3 ± 1.9	15.5 ± 1.3	60.4 ± 8.6	3.6 ± 1.2
After 1 μg	5.93 ± 0.36	0.99 ± 0.06	1.11 ± 0.13	0.00 ± 0.00	5.1 ± 0.6	7.9 ± 1.2	12.8 ± 0.8	60.9 ± 9.3	2.8 ± 0.7
After 3 mg/kg	6.03 ± 0.36	1.00 ± 0.06	1.07 ± 0.07	0.01 ± 0.01	5.2 ± 0.8	7.6 ± 1.0	14.1 ± 1.4	61.2 ± 8.4	2.4 ± 0.4
Intrathecal (6):									
Before drug	4.76 ± 0.23	0.79 ± 0.04	0.88 ± 0.04	0.00 ± 0.00	5.4 ± 1.3	8.9 ± 1.0	17.7 ± 1.0	58.5 ± 0.5	3.5 ± 0.5
After 1 μg	4.57 ± 0.14	0.76 ± 0.02	0.88 ± 0.06	0.01 ± 0.02	5.0 ± 1.0	9.1 ± 1.4	19.9 ± 0.9	63.6 ± 4.5	4.1 ± 0.9
				Obstructed					
Intravenous (6):									
Before drug	2.89 ± 0.46	0.48 ± 0.08	0.46 ± 0.08	0.05 ± 0.02	18.2 ± 1.3	27.7 ± 2.4	50.2 ± 5.8	122.4 ± 9.7	10.5 ± 1.6
After 1 μg	2.96 ± 0.56	0.49 ± 0.09	0.44 ± 0.09	0.06 ± 0.02	17.2 ± 1.7	25.6 ± 2.1	50.1 ± 5.4	120.9 ± 9.2	10.1 ± 1.4
After 3 mg/kg	5.09 ± 0.98†	0.85 ± 0.16†	0.81 ± 0.20†	0.09 ± 0.04	10.4 ± 1.9†	17.8 ± 2.4†	31.7 ± 3.6†	79.1 ± 4.7†	6.3 ± 1.1†
Intrathecal (6):									
Before drug	2.00 ± 0.18	0.33 ± 0.03	0.38 ± 0.03	0.01 ± 0.01	14.2 ± 2.2	26.8 ± 4.0	39.3 ± 3.5	83.3 ± 9.5	12.7 ± 2.5
After 1 μg	3.15 ± 0.36‡	0.53 ± 0.06‡	0.53 ± 0.07‡	0.04 ± 0.03	7.8 ± 1.2‡	11.1 ± 1.6‡	19.8 ± 1.9‡	58.5 ± 7.0‡	3.2 ± 0.6‡

* Student t test $p < 0.05$ vs nonobstructed.
† One-way repeated measurements ANOVA $p < 0.05$ vs baseline and vs 1 μg.
‡ Paired Student t test $p < 0.05$ vs baseline.

sildenafil (3 mg/kg) normalized urodynamic parameters in BO rats. However, the effect on frequency (as a surrogate for bladder afferent activity) was more pronounced than the effect on bladder pressure (as a surrogate for bladder efferent activity). The lack of a sildenafil effect on micturition in normal rats and its clear effect in BO rats suggest that the targeted NO/cGMP pathway is less relevant under normal conditions but it is involved in BO related to PUO.

Intrathecal sildenafil (1 μg) had the same urodynamic effects in obstructed rats as intravenous sildenafil (3 mg/kg). However, the same sildenafil dose given intrathecally (1 μg) had no urodynamic effect when given intravenously. This indicates that sildenafil affects urodynamic parameters via a spinal site of action, most likely the afferent branch of the micturition reflex.

Other investigators reported data suggesting effects of PDE5 inhibitors on afferent function. In an acute model of acrolein induced BO tadalafil decreased bladder afferent firing.[24] Similarly, vardenafil decreased bladder afferent nerve firing in rats with an injured spinal cord.[25] In a model of capsaicin induced BO (C-fiber activation) intravenous sildenafil and vardenafil decreased urodynamic parameters mainly associated with sensory afferent function.[26]

Inhibiting spinal PDE5 can be assumed to increase local cGMP. If this was linked to the urodynamic effects noted in our study, inhibition of the NO/cGMP pathway should be expected to have opposite effects. Intrathecal administration of L-NAME, an inhibitor of NO synthase, had no effect on BO induced by intravesical capsaicin but it decreased nociceptive behavior.[27] This is in agreement with the pain study by Schmidtko et al showing that intrathecally administered substances that inhibited the NO/cGMP pathway, such as L-NAME, the guanylyl cyclase inhibitor ODQ or protein kinase I inhibitors, had antinociceptive effects.[28] However, there was also evidence for substances that activate the NO/cGMP pathway, such as NO donors, cGMP analogues and PDE5 inhibitors, which have antinociceptive effects. Thus, spinal NO/cGMP may increase and decrease pain. Schmidtko et al suggested that this may be dose dependent, that is high levels of cGMP produce nociceptive effects and low concentrations are antinociceptive.[28]

A dual effect may also be exerted on urodynamic parameters. Pandita et al found that intrathecal L-NAME had no effect on normal micturition or capsaicin stimulated bladder activity.[27] In contrast, others reported that BO evoked by acetic acid[29] and turpentine[30] in anesthetized rats was decreased by intrathecally administered NO synthase inhibitors.

To our knowledge it remains to be determined whether urodynamic effects are linked to spinal cGMP concentrations. However, it cannot be excluded that the conflicting data on urodynamic effects involving the spinal NO/cGMP pathway can be explained by certain differences, eg the animal models and drug doses used. Such differences in experimental conditions can lead to variations in the spinal concentration of cGMP in neurons that control the micturition reflex.

CONCLUSIONS

PDE5 was expressed in various regions of the gray matter of the lumbosacral spinal cord, including the micturition control regions in the sacral dorsal horn layers. There was no difference in spinal PDE5 expression between normal rats and rats with PUO/BO. Sildenafil had no urodynamic effect in normal rats but pronounced effects in PUO/BO rats, indicating that the NO/cGMP pathway was altered and it is relevant in PUO/BO. Sildenafil administered as a small dose (1 μg) directly to the sacral spinal cord had urodynamic effects that could not be explained by a peripheral site of action. Thus, the urodynamic effects of intravenous sildenafil in PUO/BO rats may be mediated at least in part by effects on the sacral spinal cord.

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