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Perioperative Betamethasone Treatment Reduces Signs of Bladder Dysfunction in a Rat Model for Neurapraxia in Female Urogenital Surgery

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Abstract

Background—Information on autonomic neurapraxia in female urogenital surgery is scarce, and a model to study it is not available.

Objective—To develop a model to study the impact of autonomic neurapraxia on bladder function in female rats, as well as to assess the effects of corticosteroid therapy on the recovery of bladder function in this model.

Design, setting, and participants—Female Sprague-Dawley rats were subjected to bilateral pelvic nerve crush (PNC) and perioperatively treated with betamethasone or vehicle. Bladder function and morphology of bladder tissue were evaluated and compared with sham-operated rats.

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Outcome measurements and statistical analysis—Western blot, immunohistochemistry, organ bath experiments, and cystometry.

Results and limitations—Sham-operated rats exhibited regular micturitions without nonvoiding contractions (NVCs). Crush of all nerve branches of the pelvic plexus or PNC resulted in overflow incontinence and/or NVCs. Betamethasone treatment improved recovery of regular micturitions (87.5% compared with 27% for vehicle; $p < 0.05$), reduced lowest bladder pressure (8 ± 2 cm H₂O compared with 21 ± 5 cm H₂O for vehicle; $p < 0.05$), and reduced the amplitude of NVCs but had no effect on NVC frequency in PNC rats. Compared with vehicle, betamethasone-treated PNC rats had less CD68 (a macrophage marker) in the pelvic plexus and bladder tissue. Isolated bladder from betamethasone-treated PNC rats exhibited better nerve-induced contractions, contained more cholinergic and sensory nerves, and expressed lower amounts of collagen III than bladder tissue from vehicle-treated rats.

Conclusions—PNC causes autonomic neurapraxia and functional and morphologic changes of isolated bladder tissue that can be recorded as bladder dysfunction during awake cystometry in female rats. Perioperative systemic betamethasone treatment reduced macrophage contents of the pelvic plexus and bladder, partially counteracted changes in the bladder tissue, and had protective effects on micturition function.

Keywords

Model; Pelvic; Surgery; Nerve sparing; Neuropraxia; Detrusor overactivity; Corticosteroids

1. Introduction

Carcinoma of the cervix is the third most common cancer worldwide and a main cause of cancer-related death in females [1]. Radical hysterectomy (RH) is the standard treatment of early-stage cervical cancer but is associated with morbidities such as bladder dysfunction, anorectal disorders, and sexual dysfunction that affect a patient's quality of life [2]. Sensory loss, storing and voiding dysfunction, urinary incontinence, and detrusor instability are the most common functional disorders of the lower urinary tract and are reported for 85% of patients following RH [2]. Nerve-sparing RH (NSRH), which involves procedures that are suggested not to compromise oncologic radicality but to improve postoperative morbidity, including bladder dysfunction, has been introduced; however, large prospective randomized studies comparing RH with NSRH are missing [2]. Experiences from NSRP suggest that despite refined surgical approaches, damage to the pelvic autonomic nerves occurs because of stretch, compression, thermal effects, ischemia, edema, and local inflammation [3]. This damage leads to a temporary blockade of the signal function of the nerves (neurapraxia) and may also cause degeneration of nerve fibers and structural and functional alterations of penile erectile tissue [3,4]. The rat model of cavernous nerve crush injury is widely used to simulate the neurapraxia associated with NSRP, and various approaches, such as pharmacotherapy, gene therapy, or stem cell therapy, have been investigated in this model to overcome neurapraxia, reduce nerve damage, and improve functional autonomic nerve recovery [5–7]. Similar models are also used for somatosensory nerves, and crush of the rat pudendal nerve may be used for the study of the external urethral sphincter [8,9].

To the best of our knowledge, models to simulate pelvic autonomic neurapraxia associated with female urogenital surgery are not established. We aimed to develop a model to study the impact of damage to pelvic autonomic nerves on bladder function in female rats. In addition, we aimed to study the effect of perioperative corticosteroid treatment on the recovery of bladder function in this model.

2. Material and methods

2.1. Ethical approval

The Ethics Committee of San Raffaele University, Milan, Italy, approved all procedures.

2.2. Design of investigation

To assess the extent of nerve damage needed to affect bladder function (study A), rats were subjected to bilateral pelvic nerve crush (PNC) ($n = 11$) or crush of the bilateral PNC, the hypogastric nerve, and the vesicogenital branches of the pelvic plexus supplying the urinary bladder, outflow region, and vagina ("clock nerve crush" [CNC]; $n = 10$) (Fig. 1). Comparisons were made with sham-operated rats ($n = 10$). Cystometry was performed on day 3 or day 10 after nerve injury. Based on findings in study A, rats ($n = 22$) were subjected to bilateral PNC (study B) and were randomly assigned to receive betamethasone ($n = 10$) or vehicle ($n = 12$) subcutaneously 2 d before, until 2 d after, nerve injury (Fig. 2). Eight sham-operated rats functioned as controls. On day 10, cystometry were performed. Bladders were harvested and processed for histologic and functional investigations [10,11].

2.3. Animals

Normal female Sprague-Dawley rats ($n = 61$; 250 g) were used. Intraperitoneal ketamine (75 mg/kg) and xylazine (50 mg/kg) were used for anesthesia for surgical procedures. Rats were sacrificed by carbon dioxide asphyxia.

2.4. Nerve crush damage

The PNC and CNC (Fig. 1) were performed by 3×15 -s crush of nerves with a pair of microforceps, as previously described [12].

2.5. Cystometry

A polyethylene catheter was implanted in the bladder dome, and 3 d later, conscious rats underwent cystometry [13]. The following parameters were recorded: (1) lowest intravesical pressure, (2) highest intravesical pressure, and (3) area under the curve (AUC). The incidence of overflow incontinence and nonvoiding contractions (NVCs) was evaluated, as well as the frequency and amplitude of NVCs, when convenient.

2.6. Functional studies of isolated tissue

Detrusor preparations ($2 \times 1 \times 6$ mm) were dissected. Experiments were performed in aerated tissue baths (37 °C, pH 7.4), and activation of nerves was performed transmurally by electrical field stimulation with a Grass S48 stimulator (Grass Instruments, USA) [10].

2.7. Immunofluorescence

Immunofluorescence stainings for calcitonin gene-related peptide (CGRP) (1:1000; Euro-Diagnostica, Sweden), protein gene product (PGP) 9.5 (1:2000; Ultracolor, UK), vesicular acetylcholine transporter protein (VACHT) (1:2000; Phoenix Pharmaceuticals, USA), smooth muscle α -actin (1:2000; Sigma-Aldrich, USA), and CD68 (a marker for macrophages) (1:200; Abcam, UK) were evaluated [10]. Sections were processed in a Leica image system (Leica Microsystems, Italy). The percent area of the bladder wall or pelvic plexus (T100 magnification) occupied by stainings was evaluated in Image J, v.1.45 s (Wayne Rasband, USA) [14]. The ratio of VACHT cell bodies to the total number of cell bodies of the pelvic plexus was analyzed.

2.8. Western blot

Amounts of early collagen deposits (collagen type III) (1:5000; Abcam) in bladder tissue were evaluated as previously described [11].

2.9. Calculations

Incidences are given as percentages, whereas other values are given as mean plus or minus standard error of the mean. For comparisons among groups, a Fischer exact test, an analysis of variance (Student-Newman-Keuls post hoc test), an extra sum square F test, and an unpaired t test were used. All calculations are based on the number of individual animals.

3. Results

3.1. Study A: cystometry

Whereas sham-operated controls displayed regular micturition cycles without NVCs, animals subjected to nerve crush exhibited, at 3 d, overflow incontinence and/or NVCs, without regular micturition cycles (Table 1). At 10 d after nerve crush, overflow incontinence and NVCs were more common in PNC and CNC rats compared with sham-operated rats (Table 2). At 3 or 10 d (Tables 1 and 2), rats with PNC or CNC exhibited mean lowest intravesical pressures that were 5.8–7.3 cm H₂O (59–100%) and 6.3–7.7 cm H₂O (58–107%) higher than sham-operated rats, and mean highest intravesical pressures that were 37–44 cm H₂O (45–55%) and 41–44 cm H₂O (51–54%) lower than sham-operated rats. The AUC was 10.7–11.6 cm H₂O/s (84–112%) and 10.1–11.9 cm H₂O/s (79–115%) larger in PNC and CNC rats compared with sham-operated rats.

3.2. Study B

3.2.1. Animals—Two betamethasone-treated rats and one vehicle-treated rat died during the study. Body weights were 262 ± 7 g (sham; $n = 8$), 276 ± 4 g (PNC vehicle; $n = 11$), and 277 ± 2 g (PNC betamethasone; $n = 8$). Bladder weights amounted to 135 ± 12 mg (sham), 486 ± 10 mg (PNC vehicle; $p < 0.01$ compared with sham), and 285 ± 4 mg (PNC betamethasone; $p < 0.05$ compared with sham and PNC vehicle).

3.2.2. Cystometry—Sham-operated rats exhibited regular micturitions without NVCs. Eight of 11 vehicle-treated PNC rats exhibited overflow incontinence. Among PNC rats

treated with betamethasone, only one rat exhibited overflow incontinence, whereas the other seven rats exhibited regular micturitions (Table 3; Fig. 3).

All vehicle-treated rats and most of the betamethasone-treated rats displayed NVCs. The frequency of NVCs was similar in PNC rats, but the amplitude of the NVCs of vehicle-treated rats was larger than for betamethasone-treated rats (Table 3). The highest intravesical pressures were lower in PNC rats compared with sham-operated rats, whereas the lowest intravesical pressure and the AUC were higher in vehicle-treated rats compared with sham-operated rats and betamethasone-treated PNC rats (Table 3).

3.2.3. Functional studies—Carbachol produced larger contractions of bladder preparations from PNC rats treated with vehicle or betamethasone than of bladder preparations from sham-operated rats (Fig. 4). Contractions to activation of nerves were lower in bladder preparations from PNC rats treated with vehicle compared with bladder preparations from sham-operated rats or PNC rats treated with betamethasone (Fig. 4).

3.2.4. Morphology and Western blot—All PNC rats exhibited more CD68 immunoreactivity (IR) in the pelvic plexus compared with sham-operated rats. No difference in CD68 content of bladder tissue was observed between betamethasone-treated PNC rats and sham-operated rats. Vehicle-treated PNC rats had larger amounts of CD68-IR in the pelvic plexus and bladders than betamethasone-treated rats (Fig. 5). Vehicle-treated PNC rats had fewer VAcHT-IR cell bodies in the pelvic plexus than betamethasone-treated or sham-operated rats (Fig. 5). CD68-IR was located to 4',6-diamidino-2-phenylindole-positive cells of the pelvic plexus (Fig. 6) and the bladder (data not shown). By Western blot, larger amounts of collagen were detected in bladder specimens from vehicle-treated rats than in bladders from betamethasone-treated rats (Fig. 7).

Whereas vehicle-treated PNC rats had lower amounts of PGP-IR in the bladder, no difference in PGP-IR content was observed for sham-operated and betamethasone-treated PNC rats (Fig. 8). Compared with sham-operated rats, all PNC rats expressed lower amounts of VAcHT-IR and CGRP-IR nerves in the bladders, but betamethasone-treated rats had larger amounts of these nerves than vehicle-treated rats (Fig. 8). The distribution of nerves in bladders of rats treated with betamethasone appeared more homogeneous compared with vehicle-treated PNC rats, which exhibited areas that were devoid of nerves (Fig. 8).

No difference in the areas of the bladder sections that were occupied by smooth muscle α -actin-IR was detected between sham-operated rats ($35.8 \pm 2.5\%$) or PNC rats treated with vehicle ($27.8 \pm 3.0\%$) or betamethasone ($33.2 \pm 3.2\%$).

4. Discussion

This study characterizes a model for autonomic neurapraxia in female rats with related bladder dysfunction during awake cystometry. It also demonstrates that perioperative systemic treatment with betamethasone ameliorates bladder dysfunction in rats with PNC. In addition, we present evidence that betamethasone depresses the immunologic responses

in the pelvic plexus and the bladder to the nerve damage, and that this action protects the integrity and function of the bladder tissue and improves recovery of micturition.

After conventional RH, an initial hyperreflexic bladder dysfunction occurs, after which a hypotonic bladder may develop [2]. Various nerve-sparing procedures are reported to reduce early postoperative bladder dysfunction after RH as assessed by improved recovery of spontaneous voiding or bladder sensation, less postoperative need for catheterization, and less time to achieve low postvoid residual volumes [2,15–17].

In rats, ablation of the pelvic nerve has been shown to cause persistent bladder dysfunction and overflow incontinence in studies 6 wk after nerve transections [18,19]. The current investigation describes early overflow incontinence of rats subjected to crush to all nerve branches of the pelvic plexus or PNC alone, and it found that recovery at 10 d of regular micturition was similar (17–25%) for either procedure. We also noted reduced maximal intravesical pressures for all nerve crush rats that may be comparable to findings in humans after NSRH [20]. Both crush procedures seem to cause changes in bladder function that may be used to study the impact of partial nerve injury on bladder function in a female rat model, but PNC is technically more convenient.

The nerve crush injury damages the myelin sheath and the axon, but it leaves the basal lamina and Schwann cell tubes intact [8]. This situation causes proliferation of Schwann cells, rapid upregulation of cytokines and chemokines, and recruitment of circulating macrophages [21]. The initial inflammation and actions by invading immune cells that are necessary for nerve regeneration also have negative effects associated with denervation, reorganization of the extracellular matrix, delayed functional recovery, or development of nerve dysfunction [21,22]. Recent studies also identify macrophages as important activators of fibroblasts [23]. As seen in PNC rats, the contents of cholinergic motor nerves and sensory nerves of the bladder were reduced and collagen contents increased. Concomitantly, nerve-mediated contractile responses were lower in isolated bladder tissue from these animals that also exhibited bladder dysfunction *in vivo*. Our findings are comparable to morphologic changes in, and dysfunction of, isolated erectile tissue from rats that exhibit erectile dysfunction due to crush of the cavernous nerve [4,24].

Corticosteroids have beneficial effects on functional recovery after spinal cord injuries and are known to inhibit the vasodilation, vascular permeability, and edema that occur in inflammation [25,26]. Actions by corticosteroids include transcriptional repression of immune progenitor cells, reversal of cytokine responses toward anti-inflammation, reduction of macrophage migration, and inhibition of lymphocytes [25,26]. In models for sciatic or cochlear nerve crush injury, systemic perioperative treatment with corticosteroids is reported to decrease nerve damage or degeneration, with concomitant reductions of the early recruitment of macrophages [27–29]. Similarly, we demonstrate less CD68 (a macrophage marker) in bladder tissue and the pelvic plexus and more nerves and less collagen in bladder tissue from PNC rats treated with betamethasone compared with vehicle. Nerve function of isolated bladders was better in PNC rats treated with betamethasone than with vehicle. However, isolated bladders from all PNC rats exhibited muscarinic agonist supersensitivity. Even if betamethasone-treated rats had more VACHT nerves than vehicle-treated rats,

compensatory upregulation of postjunctional muscarinic receptors may be expected as a result of partial loss of bladder motor neurons in both groups compared with sham-operated rats. The idea that this contributed to preserved nerve-induced contractions of bladder preparations from betamethasone PNC rats cannot be excluded.

In agreement with the effects of betamethasone on the morphology and function of isolated bladders from PNC rats, the main finding of this study was that 87.5% of the betamethasone-treated rats, and only 27% of the vehicle-treated rats, exhibited regular micturitions. We also recorded similar lowest intravesical pressure (basal pressure) and AUC in betamethasone-treated PNC rats and sham-operated rats. Probably because of a combination of partial loss of motor nerves and change in viscous properties of the bladder due to increase in collagen, the capacity of PNC rats to generate higher pressures was reduced. This characteristic was not reversed by beta-methasone at 10 d.

In contrast to our findings, studies of the effect of corticosteroids on recovery of sexual function in men after NSRP have previously not shown promising results [3]. In these studies, systemic corticosteroid treatment was initiated on postoperative day 1 or corticosteroids were applied locally on the neurovascular bundle during surgery [3]. Considering that the inflammatory response is already active within hours after nerve injury and that recruitment of circulating immune cells occurs in the degeneration process, the timing and route of administration of corticosteroids is of importance in the anti-inflammatory effect [21,30]. In the current study, we initiated betamethasone treatment 2 d before nerve crush to ensure optimal tissue concentrations and to try to improve effects on gene repression of immune progenitor cells at the time of injury.

5. Conclusions

PNC causes autonomic neurapraxia that can be recorded as bladder dysfunction during awake cystometry in female rats. Perioperative systemic betamethasone treatment reduced macrophage contents of the pelvic plexus and bladder, partially counteracted functional and morphologic changes of isolated bladder tissue, and improved recovery of micturition function in these rats.

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Fig. 1.

Schematic illustration of the female rat pelvic organs, the pelvic plexus, and adjoining nerves: (a) the point (red circle) of crush injury of the pelvic nerve; (b) the different points (red circles) of crush injury of the pelvic nerve, hypogastric nerve, and vesicogenital branches of the pelvic plexus in the “clock nerve crush” procedure.

Fig. 2.

Design of study B to investigate the effect of perioperative systemic (subcutaneous) administration of betamethasone or vehicle on bladder function in rats subjected to bilateral pelvic nerve crush. Comparison with sham-operated rats was made.

Fig. 3.

Rat cystometry; original tracings from study B depicting intravesical pressure (in centimeters of water) and voided volume (in milliliters) from awake rats during continuous cystometry 10 d postoperatively. (a) Sham-operated rat; (b) rat subjected to pelvic nerve crush and treated with vehicle that displays overflow incontinence and nonvoiding contractions; (c) rat subjected to pelvic nerve crush and treated with betamethasone that displays regular micturitions and nonvoiding contractions. *Emptying of cup for voided volume.

Fig. 4.

Functional investigations of isolated bladder tissue from sham-operated rats or rats subjected to pelvic nerve crush (PNC) and treated with vehicle or betamethasone. (a) Concentration–response curves to carbachol (10⁻⁸–10⁻⁴ M). Indicating supersensitivity to muscarinic receptor activation, all PNC rats (vehicle: $n = 9$; betamethasone: $n = 8$) exhibit larger $E_{\max}$ to carbachol than sham-operated rats ($n = 7$). (b) Frequency–response curves to transmural activation of nerves. Vehicle-treated PNC rats ($n = 8$) exhibit lower contractions compared with responses of sham-operated rats ($n = 7$) or PNC rats treated with betamethasone ($n = 7$). Comparisons of concentration–response curve and frequency–response curve between groups were made by the extra sum square F test. ** $p < 0.01$.

Fig. 5.

Immunohistochemistry. Immunoreactivity (IR) for the macrophage surface marker CD68 in the bladders from (a) a sham-operated rat, (b) a pelvic nerve crush (PNC) rat treated with vehicle, and (c) a PNC rat treated with betamethasone. Magnification $\times 100$, Alexa green. (d) Bar graph depicting the area of the bladder wall that was occupied by CD68-IR in sections from sham-operated rats ($n = 8$) and PNC rats treated with vehicle ($n = 7$) or betamethasone ($n = 6$). (e–g) Images are the same sections as in (a–c), depicting the cellular content of the bladders by 4',6-diamidino-2-phenylindole (DAPI) stainings for nuclei. CD68-IR in the pelvic plexus is depicted in (h) from a sham-operated rat, in (i) from a PNC rat treated with vehicle, and in (j) from a PNC rat treated with betamethasone. Magnification $\times 100$, Alexa green. (k) Bar graph depicting the area of the pelvic plexus that was occupied by CD68-IR in sections from sham-operated rats ($n = 7$) and PNC rats treated with vehicle ($n = 7$) or betamethasone ($n = 6$). (l–n) Images are the same sections as in (h–j), depicting nerve cell bodies costained for the vesicular acetylcholine transporter protein (VAcHT); Alexa red. (o) Bar graph depicting the number of VAcHT-containing nerve cell bodies in relation to the total number of nerve cells (DAPI) per visual field at T200 magnification of the pelvic plexus of sham-operated rats ($n = 7$) and PNC rats treated with vehicle ($n = 7$) or

betamethasone ($n = 6$). Values of bar graphs are given as mean plus or minus standard error of the mean. Statistical comparisons were made by an analysis of variance. * $p < 0.05$ compared with sham-operated rats; § $p < 0.05$ compared with PNC rats treated with vehicle.

Fig. 6. Immunohistochemistry. Immunoreactivity for the macrophage surface marker CD68 in cells around a vesicular acetylcholine transporter protein (VACHT)-positive nerve cell body of the pelvic plexus from a pelvic nerve crush rat treated with vehicle. (a) Nerve cell body containing VACHT immunoreactivity; Alexa green. (b) Same section as in (a); CD68 immunoreactivity in cells; Alexa red. (c) Same section as in (a) and (b); nuclear 4',6-diamidino-2-phenylindole (DAPI) stainings; blue. (d) Merged image of (a–c) depicting appropriate position of nuclei of the larger VACHT-positive ganglion cell (center; Alexa green); numerous CD68-positive cells around the VACHT-positive nerve cell body. Some DAPI-positive stainings do not contain CD68 and represent other cell types. Magnification $\times 1000$, oil.

Fig. 7.

Western blot, bladder collagen III contents. (a) Representative bands for collagen III at the expected size of 130 kDa in bladders from pelvic nerve crush (PNC) rat treated with betamethasone (lanes 1–3), PNC rats treated with vehicle (lane 4–6), and bladders from sham-operated rats (lanes 7–9). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 37 kDa, was used as a control. (b) Bar graph depicting the optical density ratios of collagen III to GAPDH in sham-operated rats ($n = 6$) and in PNC rats treated with vehicle (veh) ($n = 6$) or betamethasone ($n = 6$). Statistical comparisons were made by an analysis of variance. * $p < 0.05$ compared with sham-operated rats; § $p < 0.05$ compared with PNC rats treated with vehicle.

Fig. 8.

Immunohistochemistry. (a) Immunoreactivity (IR) in nerves for protein gene product (PGP) 9.5 (a general nerve marker) of a bladder from a sham-operated rat. (b) PGP-IR in the bladder from a vehicle-treated rat subjected to pelvic nerve crush (PNC). (c) PGP-IR in the bladder from a PNC rat treated with betamethasone. Magnification $\times 100$, Alexa green. (d) Bar graph showing that smaller areas of the bladder were occupied by PGP-IR in bladders from PNC rats treated with vehicle ($n = 5$) than in bladders from rats treated with betamethasone ($n = 5$) or sham-operated rats ($n = 6$). (e) Vesicular acetylcholine transporter protein (VAcHT)-IR in a bladder section from a sham-operated rat depicting larger amounts of VAcHT-IR than in (f), a bladder section from a vehicle-treated PNC rat. (g) Larger amounts of VAcHT-IR in a bladder section from a betamethasone-treated rat subjected to PNC compared with (f). Magnification $\times 100$, Alexa green. (h) Bar graph depicting the area of the bladder that was occupied by VAcHT-IR in sections from sham-operated rats ($n = 6$) and PNC rats treated with vehicle ($n = 5$) or betamethasone ($n = 5$). (i) Calcitonin gene-related peptide (CGRP)-IR in a bladder section from a sham-operated rat depicting larger amounts of CGRP-IR than in (j), a bladder section from a vehicle-treated PNC rat. (k) Larger amounts of CGRP-IR in a bladder section from a betamethasone-treated rat subjected to PNC compared with (j). Magnification $\times 200$; Alexa green. (l) Bar graph depicting the area of the bladder that was occupied by CGRP-IR in sections from sham-operated rats ($n = 6$) and PNC rats treated with vehicle ($n = 8$) or betamethasone ($n = 7$). Values of bars graphs

are given as mean area percentage plus or minus standard error of the mean. Statistical comparisons were made by an analysis of variance. * $p < 0.05$ compared with sham-operated rats; § $p < 0.05$ compared with PNC rats treated with vehicle.

Table 1

Urodynamic parameters 3 d after nerve crush injury or sham operation

Urodynamic parameters 3 d after nerve crush/sham operation	Overflow incontinence, incidence, %	Nonvoiding contractions, incidence, %	Lowest intravesical pressure, cm H ₂ O	Highest intravesical pressure, cm H ₂ O	Area under the curve, cm H ₂ O/s
Sham, <i>n</i> = 5	0	0	7.3 ± 0.8	74.3 ± 5.9	10.4 ± 0.9
Pelvic nerve crush, <i>n</i> = 5	100 ^{§§}	100 [§]	14.6 ± 1.8 [*]	35.3 ± 3.3 [*]	22.0 ± 1.9 [*]
Clock nerve crush, <i>n</i> = 5	100 ^{§§}	100 [§]	15.0 ± 2.0 [*]	38.2 ± 5.0 [*]	22.3 ± 2.0 [*]

Overflow incontinence is leakage of bladder content without any regular micturition cycles. Nonvoiding contractions were defined as continuously occurring increases (>2 cm H₂O) in intravesical pressure. Lowest intravesical pressure is the lowest recorded pressure in the bladder, which in sham-operated rats is equal to basal pressure. Highest intravesical pressure is the highest recorded pressure in the bladder, which in sham-operated rats is equal to maximum micturition pressure. The area under the curve was analyzed during a 20-min period at any time in nerve crush rats and in between micturitions in sham-operated rats during a similar period. Incidence values are given as percentages, and pressure parameters are given as mean plus or minus standard error of the mean.

^{§§} $p < 0.01$ (Fischer exact test compared with sham).

[§] $p < 0.05$.

^{*} For comparisons between all groups, $p < 0.05$ (analysis of variance).

Table 2

Urodynamic parameters 10 d after nerve crush injury or sham operation

Urodynamic parameters 10 d after nerve crush/sham operation	Overflow incontinence, incidence, %	Nonvoiding contractions, incidence, %	Lowest intravesical pressure, cm H ₂ O	Highest intravesical pressure, cm H ₂ O	Area under the curve, cm H ₂ O/s
Sham, <i>n</i> = 5	0	0	8.8 ± 1.2	79.8 ± 9.8	11.5 ± 0.7
Pelvic nerve crush, <i>n</i> = 6	83 ^{\$}	100 ^{\$\$}	15.5 ± 1.4 [*]	44.9 ± 7.3 [*]	23.5 ± 4.3 [*]
Clock nerve crush, <i>n</i> = 5	75 ^{\$}	100 ^{\$}	16.0 ± 3.2 [*]	38.0 ± 5.6 [*]	22.9 ± 6.0 [*]

Overflow incontinence is leakage of bladder content without any regular micturition cycles. Nonvoiding contractions were defined as continuously occurring increases (>2cm H₂O) in intravesical pressure. Lowest intravesical pressure is the lowest recorded pressure in the bladder, which in rats with regular micturitions is equal to basal pressure. Highest intravesical pressure is the highest recorded pressure in the bladder, which in sham-operated rats is equal to maximum micturition pressure. The area under the curve was analyzed during a 20-min period at any time in nerve crush rats and in between micturitions in sham-operated rats during a similar period. Incidence values are given as percentages, and pressure parameters are given as mean plus or minus standard error of the mean.

^{\$\$} *p* < 0.01 (Fischer exact test compared with sham).

^{\$} *p* < 0.05.

^{*} For comparisons between all groups, *p* < 0.05 (analysis of variance).

Table 3

Urodynamic parameters 10 d after pelvic nerve crush injury in rats treated perioperatively with vehicle or betamethasone

Urodynamic parameters 10 d after nerve crush	Overflow incontinence, incidence, %	Nonvoiding contractions, incidence, %	Nonvoiding contractions, no./min	Nonvoiding contractions, amplitude, cm H ₂ O	Lowest intravesical pressure, cm H ₂ O	Highest intravesical pressure, cm H ₂ O	Area under the curve, cm H ₂ O/s
Sham, <i>n</i> = 8	0	0	-	-	9.2 ± 1.1	86.1 ± 4.1	11.9 ± 0.9
Vehicle, <i>n</i> = 11	73 [§]	100 [§]	6.1 ± 0.5	11.3 ± 2.0	20.8 ± 5.5 [*]	55.6 ± 8.5 [*]	29.6 ± 6.5 [*]
Betamethasone, <i>n</i> = 8	12.5	75 [§]	6.2 ± 1.4	4.3 ± 1.6 ^Y	8.1 ± 1.9	45.2 ± 6.7 [*]	15.6 ± 3.4

Overflow incontinence is leakage of bladder content without any regular micturition cycle. Nonvoiding contractions were defined as continuously occurring increases (>2cm H₂O) in intravesical pressure. Lowest intravesical pressure is the lowest recorded pressure in the bladder, which in rats with regular micturitions is equal to basal pressure. Highest intravesical pressure is the highest recorded pressure in the bladder, which in rats with regular micturitions is equal to maximum micturition pressure. The area under the curve was analyzed during a 20-min period at any time in rats with dribbling incontinence and between micturitions during a similar period in rats with regular micturition. Incidence values are given as percentages, and pressure parameters are given as mean plus or minus standard error of the mean.

[§] $p < 0.05$ (Fischer exact test compared with sham).

^{*} For comparisons between all groups, $p < 0.05$ (analysis of variance).

^Y $p < 0.05$ (t test compared with vehicle).