Mechanisms of reflex bladder activation by pudendal afferents

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Wook JP, Yoo PB, Grill WM. Mechanisms of reflex bladder activation by pudendal afferents. Am J Physiol Regul Integr Comp Physiol 300: R398–R407, 2011. First published November 10, 2010; doi:10.1152/ajpregu.00154.2010.—Activation of pudendal afferents can evoke bladder contraction or relaxation dependent on the frequency of stimulation, but the mechanisms of reflex bladder excitation evoked by pudendal afferent stimulation are unknown. The objective of this study was to determine the contributions of sympathetic and parasympathetic mechanisms to bladder contractions evoked by stimulation of the dorsal nerve of the penis (DNP) in α-chloralose anesthetized adult male cats. Bladder contractions were evoked by DNP stimulation only above a bladder volume threshold equal to 73 ± 12% of the distension-evoked reflex contraction volume threshold. Bilateral hypogastric nerve transection (to eliminate sympathetic innervation of the bladder) or administration of propranolol (a β-adrenergic antagonist) decreased the stimulation-evoked and distension-evoked volume thresholds by −25% to −39%. Neither hypogastric nerve transection nor propranolol affected contraction magnitude, and robust bladder contractions were still evoked by stimulation at volume thresholds below the distension-evoked volume threshold. As well, inhibition of distension-evoked reflex bladder contractions by 10 Hz stimulation of the DNP was preserved following bilateral hypogastric nerve transection. Administration of phentolamine (an α-adrenergic antagonist) increased stimulation-evoked and distension-evoked volume thresholds by 18%, but again, robust contractions were still evoked by stimulation at volumes below the distension-evoked threshold. These results indicate that sympathetic mechanisms contribute to establishing the volume dependence of reflex contractions but are not critical to the excitatory pudendal to bladder reflex. A strong correlation between the magnitude of stimulation-evoked bladder contractions and bladder volume supports that convergence of pelvic afferents and pudendal afferents is responsible for bladder excitation evoked by pudendal afferents. Further, abolition of stimulation-evoked bladder contractions following administration of hexamethonium bromide confirmed that contractions were generated by pelvic efferent activation via the pelvic ganglion. These findings indicate that pudendal afferent stimulation evokes bladder contractions through convergence with pelvic afferents to increase pelvic efferent activity. cat; micturition; parasympathetic; sympathetic depending on the stimulation frequency, electrical stimulation of pudendal afferents evokes spinal reflexes that either inhibit the bladder and promote continence or excite the bladder and cause micturition in both cats (5, 6, 41, 45, 49) and persons with spinal cord injury (48). Previous results suggest that bladder inhibition by pudendal afferent stimulation arises from activation of hypogastric efferents and subsequent synaptic and ganglionic inhibition of parasympathetic efferents (8, 30), but the mechanisms of bladder excitation by pudendal afferent stimulation are not known. Contraction of the bladder by pudendal afferent stimulation may result from activation of a vestigial reflex from perigenital afferents to the bladder (12) or activation of an augmenting reflex from urethral afferents to the bladder (3, 36). The objective of this study was to determine the contribution of sympathetic and parasympathetic mechanisms to the reflex activation of the bladder evoked by stimulation of pudendal afferents.

Previous data suggest that suppression of the tonic sympathetic inhibition of the bladder is responsible for reflex excitation of the bladder by pudendal afferent stimulation. Sympathetic efferent activity inhibits the bladder via α-adrenergic receptor-mediated inhibition at the vesical ganglia and β-adrenergic receptor-mediated direct inhibition of the detrusor muscle (13). The sympathetic (i.e., hypogastric) reflex response to pudendal afferent stimulation depends strongly on stimulation frequency. Low-frequency pudendal afferent stimulation evokes robust reflex activation of hypogastric efferents. However, as the frequency of pudendal afferent stimulation is increased, reflex activation of hypogastric efferents declines and stimulation suppresses ongoing intrinsic hypogastric activity (30). Similarly, the bladder response to pudendal afferent stimulation exhibits a parallel dependence on the stimulation frequency; stimulation at 5–10 Hz inhibits the bladder and promotes continence, while stimulation at 33–40 Hz excites the bladder and causes voiding (6, 42, 45). The correlation between the stimulation frequency tuning of pudendal afferent-evoked excitation of the bladder and pudendal afferent-evoked suppression of hypogastric efferents suggests that reduction of the tonic inhibitory sympathetic activity (i.e., inhibiting the inhibitor) is a potential mechanism underlying reflex bladder contraction evoked by pudendal afferent stimulation.

Alternatively, reflex bladder excitation evoked by pudendal afferent stimulation may be due to convergent reflex activation of parasympathetic bladder efferents (i.e., exciting the exciter). Pudendal afferent stimulation evokes bladder contraction only when the bladder volume is above a threshold volume (~70–80% of the volume at which distension-evoked reflex bladder contractions occur) (4, 45), and this volume dependence is mediated by a neural rather than a biomechanical mechanism (4). Similarly, the magnitude of the pelvic efferent reflex response evoked by pudendal afferent stimulation increases with increased pelvic afferent activity (bladder pressure) (32). The similarity between the pelvic afferent (bladder pressure) dependence of pudendal afferent-evoked pelvic efferent activity and pudendal afferent-evoked bladder contractions suggests that convergence of somatic (pudendal) and parasympathetic (pelvic) afferents may underlie pudendal afferent-evoked activation of the bladder.

The results of the present study determine the contributions of sympathetic and parasympathetic mechanisms to bladder contractions evoked by stimulation of pudendal afferents in α-chloralose anesthetized male cats. While sympathetic activity contributed to determining the volume thresholds for stimulation-evoked and distension-evoked bladder contractions,
pharmacological block or surgical interruption of the sympathetic innervation of the bladder did not influence the magnitude of the bladder contractions evoked by stimulation of pudendal afferents. Further, a strong correlation was observed between the magnitudes of pudendal afferent stimulation-evoked bladder contractions and bladder volume, indicating that convergence of pelvic afferents and pudendal afferents is responsible for bladder excitation evoked by pudendal afferents.

MATERIALS AND METHODS

Experiments were conducted on 10 sexually intact, male cats weighing 3.3–4.5 kg. All animal care and experimental procedures were approved by the Duke University Institutional Animal Care and Use Committee and were in accord with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Anesthesia was induced with ketamine HCl (35 mg/kg im) and maintained with α-chloralose (65 mg/kg iv supplemented at 15 mg/kg as needed). The end-tidal CO₂ was maintained between 3.0 and 4.0% with artificial respiration, core body temperature was maintained at ~38°C with a thermostatic heating pad, and blood pressure was monitored through a catheter in the carotid artery. Intravenous fluids (lactated Ringer’s solution or saline/5% dextrose/sodium bicarbonate solution) were administered at 15 ml·kg⁻¹·h⁻¹ through the cephalic vein. The bladder was accessed through a midline abdominal incision. In all cats, a 3.5 French catheter was inserted into the bladder dome and secured with a purse-string suture, and the catheter was then connected to a solid-state pressure transducer (Deltran, Utah Medical) to record the bladder pressure. The catheter was also connected to a syringe for infusion of room temperature saline into the bladder. Anesthesia was induced with ketamine HCl (35 mg/kg im) and maintained with α-chloralose (65 mg/kg iv supplemented at 15 mg/kg as needed). The end-tidal CO₂ was maintained between 3.0 and 4.0% with artificial respiration, core body temperature was maintained at ~38°C with a thermostatic heating pad, and blood pressure was monitored through a catheter in the carotid artery. Intravenous fluids (lactated Ringer’s solution or saline/5% dextrose/sodium bicarbonate solution) were administered at 15 ml·kg⁻¹·h⁻¹ through the cephalic vein. The bladder was accessed through a midline abdominal incision. In seven cats, a 3.5 French catheter was inserted into the bladder dome and secured with a purse-string suture, and the catheter was then connected to a solid-state pressure transducer (Deltran, Utah Medical) to record the bladder pressure. The catheter was also connected to a syringe for infusion of room temperature saline into the bladder. The abdominal incision was closed in layers. In all cats, a 3.5 or 5 French catheter was inserted into the urethra via the urethral meatus to occlude the urethra and prevent bladder voiding. The urethral catheter was connected to a syringe and used for instilling saline into the bladder in the three cats without suprapubic catheters. Whether filling was performed through the suprapubic or urethral catheter did not appear to impact the results.

In one cat, the spinal cord was transected at the T10 vertebral level. The cord was exposed via laminectomy, the dura was incised, and lidocaine was administered to the exposed cord. The spinal cord was elevated and transected, and Surgicel was packed between the transected ends of the spinal cord.

Nerve access and stimulation. An incision was made from the skin around the prepuc to the caudal border of the gracilis muscle. The DNP was dissected free (unilaterally) from the body of the penis at the proximal end of the penile body, just distal to the bulb of the penis. A monopolar cuff electrode consisting of a platinum contact embedded in a silicone elastomer cuff was placed around the nerve, and a subcutaneous needle was inserted in the ipsilateral leg for the return electrode. Stimulation consisted of trains of constant-current (50–750 μA) 100-μs stimulation pulses.

The hypogastric nerves were accessed via a midline abdominal incision and, where indicated, were transected distal to the inferior mesenteric ganglia. Control filling trials were conducted in advance of hypogastric nerve transection but after surgical exposure of the nerve to prevent colon manipulation during nerve exposure from affecting the comparison of control and nerve transection trials. Hypogastric nerve transection was conducted in five cats: one before any other treatment, two after prior administration and washout of phentolamine and propranolol (see Drug administration), one after prior administration and washout of propranolol only, and one after prior administration and washout of phentolamine only. As well, in four of these cats, both phentolamine and propranolol were administered after bilateral hypogastric nerve transection, and these data were treated separately from the analysis of the effects of phentolamine and propranolol in isolation.

Drug administration. Drugs were administered intravenously via a catheter in the cephalic vein. Propranolol HCl (Bedford Laboratories), a β-adrenergic antagonist, and phentolamine HCl (Sigma-Aldrich), an α-adrenergic antagonist, were administered at 1 mg/kg and 2 mg/kg, respectively (10, 16, 17, 28, 37). Heart rate and arterial blood pressure were monitored to establish the onset of drug effects. As well, the adrenergic agonists phenylephrine HCl (α-agonist; Parenta Pharmaceuticals) and isoproterenol HCl (β-agonist, Sigma-Aldrich) were administered intravenously (at 30 and 50 μg/kg, respectively) before and after administration of phentolamine and propranolol, respectively, to confirm the effects of the antagonists. Hexamethonium bromide (Sigma-Aldrich) was administered intravenously at 1 mg/kg in three cats to investigate the effect of ganglionic block on the bladder contractions evoked by DNP stimulation.

Phentolamine was administered in six cats: in four cats before any other treatment; administered a second time after washout in two cats; and in two cats after administration and washout of propranolol. Propranolol was administered in six cats: in four cats before any other treatment; administered a second time after washout in two cats; and in two cats after administration and washout of phentolamine.

Determining volume thresholds. Intermittent electrical stimulation of the DNP during bladder filling was used to determine the volume thresholds for stimulation-evoked bladder contractions and distention-evoked reflex bladder contractions. Starting with an empty bladder, the bladder volume was increased by infusion of a 1-ml bolus of room temperature saline every 1 min through the urethral or suprapubic catheter. Stimulation at 33 Hz for 20 s was applied 20 s after injecting each bolus. Stimulation intensity was fixed at two times the threshold to evoke a reflex EMG response in the external anal sphincter. Trials were ended several boluses after the appearance of robust distension-evoked reflex contractions. At least 15 min, with the bladder empty, elapsed between consecutive filling trials, and a minimum of two control bladder filling trials preceded drug administration. Experimental trials were initiated 15–20 min following drug administration and were not continued beyond 90 min following drug administration. At least 2 h elapsed following drug administration before establishing new controls for subsequent drug administration or nerve transection. Threshold volumes for stimulation-evoked and distention-evoked bladder contractions (the primary outcome variables to assess the effects of adrenergic receptor blockade and hypogastric nerve transection) were remarkably stable during the course of the experiment. Across four animals in which control volumes were reestablished repeatedly between administration of propranolol and phentolamine, stimulation-evoked volume thresholds varied by only 0–3 ml (mean = 1 ml) and distention-evoked volume thresholds varied by 0–13 ml (mean = 4.75 ml). Stimulation-evoked inhibition of distention-evoked reflex bladder contractions was investigated at the end of filling trials in four animals by stimulating during contractions at 10 Hz with an amplitude equal to three times the threshold to evoke a reflex EMG response in the external anal sphincter.

Data analysis. The bladder response to electrical stimulation was analyzed by comparing the bladder pressure before and during DNP stimulation. The average bladder pressure during the 3 s prior to stimulation onset was defined as the baseline pressure, and a stimulation-evoked contraction was determined to have occurred if the bladder pressure increased by > 10 cmH₂O during the first 8 s of stimulation and was maintained until the end of stimulation. Distention-evoked reflex contractions were defined as transient rises in bladder pressure (> 10 cmH₂O compared with the prebolus bladder pressure) occurring between 5 and 20 s after injecting a 1-ml bolus. Threshold pressures for both distention-evoked and stimulation-evoked bladder contractions were determined by averaging the pressure over three seconds before bolus injection or stimulation delivery at the respective threshold volumes. Bladder inhibition was defined as a > 10 cmH₂O decrease in bladder pressure within the first 8 s of

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stimulation. The mean and maximum bladder contraction magnitudes were determined for the contraction evoked by stimulation at volumes 1 ml less than the distension-evoked contraction volume threshold. Mean stimulation-evoked contraction magnitudes were determined by averaging the bladder pressure from the onset of contraction (>10 cmH2O increase in pressure) until the termination of stimulation. The maximum contraction magnitude was defined as the maximum bladder pressure during stimulation. Distension-evoked contraction magnitudes were quantified for the contractions occurring at volumes 2 ml above the distension-evoked threshold volume by calculating the average bladder pressure for the 15 s prior to the onset of DNP stimulation.

Experimental trials were grouped with the control trials that preceded those experimental trials, and the volume thresholds were normalized by dividing the threshold volumes by the average of the control distension-evoked threshold volumes. The normalized values were averaged for each cat and compared for statistically significant differences using ANOVA with post hoc paired comparisons using Bonferroni correction. Contraction magnitudes (mean and maximum) were normalized for each set of control and experimental trials by dividing each magnitude by the average of the control magnitudes. Normalized contraction magnitudes were compared using an unpaired, two-tailed t-test. Bladder inhibition was quantified by taking the ratio of the average bladder pressure for the final 10 s of 10 Hz DNP stimulation and the average pressure for 3 s prior to the onset of the distension-evoked contraction. Ratios before and after sympathetic block were compared using a paired, two-tailed t-test. All reported values are means ± SD.

RESULTS

Volume thresholds for distension-evoked and stimulation-evoked contractions. High-frequency (33 Hz) stimulation of pudendal afferents in the DNP evoked bladder contractions in 10 of 10 cats. In nine cats, the bladder volume thresholds for distension-evoked reflex bladder contractions (distension-evoked threshold volume (DTV)) and the bladder volume thresholds for stimulation-evoked contractions (stimulation-evoked threshold volume (STV)) were determined during bladder filling in 1-ml increments (Fig. 1A). Stimulation-evoked bladder contractions during control filling (in the absence of sympathetic block) were evoked at STVs (mean: 18 ± 9 ml, range: 5–33 ml, n = 9 cats) that were 74 ± 11% of the DTVs (mean ± SD: 24 ± 12, range: 8–42 ml).

Similarly, threshold pressures were lower for stimulation-evoked bladder contractions (16 ± 3 cmH2O) than threshold pressures for distension-evoked bladder contractions (18 ± 4 cmH2O, P < 0.001). Mean (29 ± 10 cmH2O, n = 9 cats) and maximum (55 ± 15 cmH2O) stimulation-evoked contraction magnitudes were determined at bladder volumes 1 ml less than DTV, and mean (10 ± 5 cmH2O) and maximum (40 ± 28 cmH2O) distension-evoked bladder contraction magnitudes were determined at bladder volumes 2 ml greater than DTV.

Blockade of β-adrenergic receptors decreased volume thresholds. Bladder filling and stimulation were repeated following administration of the β-adrenergic antagonist propranolol (1 mg/kg) in six cats. Bladder contractions were still evoked by DNP stimulation and distension following propranolol administration (Fig. 2A), but volume thresholds were significantly reduced (Fig. 2B). After propranolol administration, stimulation-evoked bladder contractions were evoked at lower volumes (STV: 11 ± 7 ml, range: 4–21 ml, n = 6 cats) than under control conditions (STV: 18 ± 11 ml, range: 5–27 ml), and propranolol similarly decreased threshold volumes for distension-evoked contractions (DTV: 18 ± 12 ml, range: 7–39 ml) compared with control (DTV: 24 ± 15 ml, range: 8–43 ml). Thus, after propranolol administration, bladder contractions were still evoked by stimulation at volumes less than the threshold for distension-evoked reflex contractions (63 ± 19% of DTV, compared with 70 ± 11% under control conditions). Stimulation-evoked contraction magnitudes after propranolol (mean: 32 ± 9 cmH2O, maximum: 63 ± 10 cmH2O) were similar to those evoked in control conditions (mean: 30 ± 9 cmH2O, maximum: 57 ± 14 cmH2O) (Fig. 2C). Similarly, distension-evoked contraction magnitudes were not affected by propranolol administration (Fig. 2C). As well, propranolol administration did not influence threshold pressures for distension-evoked (20 ± 3 cmH2O, P = 0.35) or stimulation-evoked (16 ± 4 cmH2O, P = 0.16) contractions. Propranolol administration blocked the transient increase in heart rate evoked by the β-adrenergic agonist isoproterenol (50 μg/kg iv; 41 ± 7 beats/min increase in the absence of propranolol vs. 1.5 ± 0 beats/min in the presence of propranolol), confirming the effectiveness of the drug and dose (n = 3 cats).
Blockade of α-adrenergic receptors increased volume thresholds and decreased contraction magnitudes. Following administration of the α-antagonist phentolamine (2 mg/kg), bladder contractions were still evoked by DNP stimulation and distension in six of six cats (Fig. 3A), but volume thresholds were significantly increased (Fig. 3B). After phentolamine, STVs (mean: 26 ± 5 ml, range: 18–31 ml, n = 6 cats) and DTVs (mean: 33 ± 8 ml, range: 21–44 ml) were both increased compared with controls (STVs: 22 ± 5 ml, range: 15–28 ml; DTVs: 28 ± 8 ml, range: 17–37 ml), and stimulation still evoked bladder contractions at volumes below the DTV (STVs were 81 ± 8% of DTVs after phentolamine compared with 79 ± 13% under control conditions). However, stimulation-evoked contraction magnitudes were smaller after phentolamine (mean: 21 ± 9 cmH2O, maximum: 41 ± 16 cmH2O) compared with control contraction magnitudes (mean: 28 ± 11 cmH2O, maximum: 53 ± 16 cmH2O) (Fig. 3C). The distension-evoked contraction magnitudes following phentolamine were also smaller than under control conditions, but the difference was not significant (Fig. 3C). Phentolamine administration did not influence threshold pressures for distension-evoked contractions (20 ± 6 cmH2O, P = 0.20) but did cause a small increase in threshold pressures for stimulation-evoked contractions (18 ± 3 cmH2O, P = 0.02). Phentolamine administration blocked the transient increase in arterial pressure caused by administration of the α-agonist phenylephrine (30 μg/kg iv; 64 ± 8 mmHg increase in the absence of phentolamine vs. 2 ± 1 mmHg increase in the presence of phentolamine, n = 3 cats), verifying the effectiveness of the dose.

Hypogastric nerve transection decreased volume thresholds. Bladder contractions were still evoked by DNP stimulation and distension following bilateral transection of the hypogastric nerve in five of five cats (Fig. 4A), but threshold volumes were decreased (Figs. 1B and 4B). Hypogastric nerve transection caused a decrease in STVs (control: 16 ± 11 ml, n = 5 cats; transection: 11 ± 9 ml) and DTVs (control: 22 ± 13 ml; transection: 16 ± 11 ml), but STVs remained lower (72 ± 16%) than DTVs after hypogastric transection, comparable to the relative volume thresholds under control conditions (75 ± 10%). Bilateral hypogastric transection did not cause significant changes in the mean (30 ± 14 cmH2O) or maximum (59 ± 23 cmH2O) magnitude of stimulation-evoked bladder contractions compared with control contraction magnitudes (mean: 31 ± 13 cmH2O, maximum: 59 ± 21 cmH2O) (Fig. 4C) or in the threshold pressures for distention-evoked (17 ± 6 cmH2O, P = 0.43) or stimulation-evoked (14 ± 5 cmH2O, P = 0.08) contractions. The bladder volume thresholds and stimulation-evoked contraction magnitudes following hypogastric transection were not affected by subsequent administration of both propranolol and phentolamine (four cats) (Fig. 4, B and C). Distension-evoked contraction magnitudes were also not affected by hypogastric nerve transection or subsequent drug administration (Fig. 4C).

Hexamethonium bromide abolished stimulation-evoked contractions. In two of two cats, administration of hexamethonium bromide (1 mg/kg) following bilateral hypogastric

Fig. 2. Effect of propranolol administration on bladder contractions evoked by DNP stimulation or distension. A: example bladder responses evoked by 33 Hz DNP stimulation before and after propranolol administration (1 mg/kg). The contractions were evoked during bladder filling trials at bladder volumes 1 ml less than the distension-evoked threshold volume. B: normalized volume thresholds before and after propranolol administration were significantly different (P < 0.01, ANOVA, n = 6 cats). *Significant difference between stimulation-evoked and distension-evoked volume thresholds for control trials or for propranolol trials (P < 0.05, post hoc Bonferroni comparisons). †Significant difference between control and propranolol stimulation volume thresholds or for distension volume thresholds (P < 0.05, post hoc Bonferroni comparisons). C: normalized mean contraction magnitudes evoked by stimulation before and after propranolol were not different (P = 0.15, t-test, n = 6 cats). Also, distension-evoked contraction magnitudes increased slightly following propranolol, but the difference compared with control distension-evoked contractions was not significant (P = 0.28).
Fig. 3. Effect of phentolamine administration on bladder contractions evoked by DNP stimulation or distension. A: contractions evoked at bladder volumes 1 ml less than the distension-evoked threshold volumes by 33 Hz DNP stimulation before and after phentolamine (2 mg/kg). B: normalized stimulation-evoked and distension-evoked reflex bladder contraction volume thresholds before and after phentolamine were significantly different (P < 10^{-3}, ANOVA, n = 6 cats). †Significant difference between stimulation-evoked and distension-evoked volume thresholds for control trials or for phentolamine trials (P < 0.05, post hoc Bonferroni comparisons). *Significant difference between control and phentolamine stimulation volume thresholds or for distension volume thresholds (P < 0.05, post hoc Bonferroni comparisons). C: normalized mean contraction magnitudes evoked by 33 Hz DNP stimulation before and after phentolamine (*P < 0.02, t-test, n = 6 cats). Distension-evoked contractions decreased after phentolamine, but the difference in relative contraction magnitudes was not significant (P = 0.34).

Fig. 4. Effect of bilateral hypogastric nerve transection and subsequent adrenergic antagonist administration on bladder contractions evoked by DNP stimulation or distension. A: contractions evoked by 33 Hz DNP stimulation at a volume 1 cc less than the distension-evoked threshold volumes before and after bilateral hypogastric nerve transection. B: normalized volume thresholds before and after hypogastric nerve transection and after subsequent propranolol and phentolamine administration. Volume thresholds were significantly different (P < 10^{-12}, ANOVA, n = 36 trials). *Stimulation-evoked volume thresholds were significantly less than distension-evoked thresholds for the treatment group (P < 0.05, post hoc Bonferroni comparisons). †Significant difference between treatment and control volume thresholds for stimulation thresholds or for distension thresholds (P < 0.05, post hoc Bonferroni comparisons). C: normalized mean contraction amplitudes evoked by DNP stimulation before and after hypogastric nerve transection and subsequent drug administration were not significantly altered by hypogastric nerve transection or subsequent drug administration (P = 0.35). Normalized distension-evoked contraction magnitudes also were not significantly altered by hypogastric nerve transection or subsequent drug administration (P = 0.96).
nerve transection abolished the bladder contractions evoked by DNP stimulation (Fig. 5). The bladder contractions evoked by stimulation returned over time as the ganglionic block diminished. In one cat, the spinal cord was transected at T10 and bladder contractions were evoked by DNP stimulation 6 h following spinal cord transection. Administration of hexamethonium bromide abolished the bladder contractions evoked by stimulation following spinal cord transection.

Stimulation-evoked contraction magnitudes increased with increasing bladder volume. In three cats, the mean and maximum contraction magnitudes evoked by DNP stimulation across a range of bladder volumes were used to determine the relationship between bladder volume and contraction magnitude (Fig. 6). Regression analysis revealed a strong, positive correlation between the mean or maximum contraction magnitude and the bladder volume, and this strong, positive cor-

Fig. 6. The magnitude of bladder contractions evoked by DNP stimulation were strongly correlated with bladder volume before (A) and after (B) bilateral hypogastric nerve transection. The magnitudes of DNP stimulation-evoked bladder contractions were determined at 1-cc volume intervals between the stimulation-evoked contraction volume threshold and suprathreshold volumes. Contraction magnitudes were normalized by scaling between 0 (minimum value) and 1 (maximum value). A, left: normalized mean contraction pressures as a function of normalized bladder volume; right: regression lines (linear or quadratic) for the relationship between bladder volume and mean contraction magnitude for each cat. A significant correlation was found between mean contraction magnitude and bladder volume for all 3 cats investigated ($P < 0.0001$, Cat 1: $r = 0.8518$, $n = 3$; Cat 2: $r = 0.8480$, $n = 7$; Cat 3: $r = 0.8708$, $n = 4$). B, after bilateral hypogastric nerve transection. Left: normalized maximum contraction magnitudes as a function of normalized bladder volume. Right: regression lines (linear or quadratic) for the relationship between bladder volume and maximum contraction magnitude for each cat. A significant correlation was found between maximum contraction magnitude and bladder volume for all 3 cats investigated ($P < 0.0001$, Cat 1: $r = 0.8893$, $n = 3$; Cat 2: $r = 0.8687$, $n = 7$; Cat 3: $r = 0.8688$, $n = 4$).
relation persisted following bilateral hypogastric nerve transection.

Bladder inhibition persisted following bilateral hypogastric nerve transection. Stimulation of the DNP at 10 Hz inhibited distension-evoked reflex bladder contractions in four of four cats. Following hypogastric transection (4 cats) and hypogastric transection plus administration of phentolamine and propranolol (3 cats), 10 Hz DNP stimulation continued to evoke robust bladder inhibition (Fig. 7A). The reductions in bladder pressure (relative to baseline) evoked by 10 Hz stimulation following bilateral hypogastric nerve transection were identical to the reductions evoked under control conditions (Fig. 7B).

DISCUSSION

The mechanisms of reflex bladder excitation by activation of pudendal afferents were investigated using a combination of selective pharmacological sympathetic block and sympathetic block via hypogastric nerve transection. The sympathetic bladder innervation did not play a significant role in the reflex activation of the bladder evoked by pudendal afferent stimulation, but did play a pivotal role in determining the threshold volumes for stimulation-evoked and distension-evoked reflex bladder contractions. These results indicate that suppression of sympathetic efferent activity in the hypogastric nerve by pudendal nerve stimulation (i.e., inhibiting the inhibitor) is not the mechanism for bladder contractions evoked by pudendal afferent stimulation, but rather imply that convergence of pelvic and pudendal afferents (driving pelvic efferent activity) is responsible for bladder excitation evoked by pudendal afferents. This alternative hypothesis was supported by the lower volume threshold for stimulation-evoked bladder contractions compared with distension-evoked bladder contractions and the strong positive correlation between bladder volume (i.e., increased pelvic afferent activity) and the magnitude of stimulation-evoked bladder contractions. Further, the abolition of stimulation-evoked bladder contractions following administration of hexamethonium bromide confirmed that contractions were generated by pelvic efferent activation in the pelvic ganglion and not through an alternative pathway (e.g., somatic muscle contraction). These findings indicate that pudendal afferent stimulation evokes bladder contractions through convergence with pelvic afferents to increase pelvic efferent activity (i.e., exciting the exciter).

Selective pharmacological block of sympathetic activity revealed the importance of the sympathetic bladder innervation in determining the volume thresholds for distension-evoked and stimulation-evoked contractions. While complete block of adrenergic bladder innervation with chemical antagonists cannot be guaranteed, phentolamine and propranolol were administered at doses previously found to have maximal effects on bladder and urethral function (10, 16, 17, 28, 37). Additionally, although adrenergic agonists were administered to confirm antagonist effectiveness, secondary effects of the drugs may have altered the bladder activity. For example, adrenergic antagonists block sympathetic inhibition of the colon in the cat (19), and changes in intracolonic pressure can modulate bladder parasympathetic activity (32). As well, increases in β-adrenergic tone triggered by reductions in blood pressure following α-adrenergic blockade with phentolamine may have contributed to the observed increases in bladder volume thresholds. For example, hypogastric nerve activity augments bladder inhibition generated by pudendal afferent stimulation (42), while, as shown here, blocking the β-component decreases bladder volume thresholds. Importantly, this secondary effect does not influence the conclusion that α- or β-receptor-mediated sympathetic components are not critical to DNP stimulation-evoked bladder contractions.

β-adrenergic block. Pharmacological block of the sympathetic pathways innervating the urinary bladder illustrated the importance of the β-adrenergic receptors in determining the volume thresholds for distension-evoked and stimulation-evoked contractions. Blocking the β-adrenergic bladder pathway with propranolol reduced volume thresholds but did not affect the magnitude of the electrically evoked bladder contraction. The reduction in distension-evoked volume threshold corroborates previous studies in the dog (35) and cat (16), both of which express β1- and β2-receptor subtypes (1, 33, 34). However, propranolol did not alter the micturition thresholds in the rat (31), which expresses the β3-receptor subtype (15, 47), underscoring both important species differences as well as the substantially higher affinity of propranolol for β1- and β2-receptor subtypes (21). The results indicate that β-adrenergic-mediated bladder inhibition does not play an important role in reflex excitation of the bladder by pudendal afferent stimu-
lation but does play a major role in determining the volume thresholds.

**α-Adrenergic block.** Blocking the α-adrenergic pathway with phentolamine increased the threshold volumes for distention-evoked and stimulation-evoked contractions. Although similar increases in the micturition volume threshold following α-adrenergic block were reported in the decerebrate dog (35) and conscious rat (43), others have reported decreases in the micturition volume threshold in cats (16, 18). One factor potentially responsible for these differences is peripheral (35, 43) vs. central (18) drug administration (25). As well, the results may reflect variation in the α-adrenergic receptor subtypes, as intrathecal injection of prazosin increased the micturition volume threshold in rats (25) but had little effect in cats (18). Further, differences in anesthesia across these studies may have influenced the effects of phentolamine. The current study was conducted under α-chloralose anesthesia, which affects spinal reflexes (40) and bladder behavior (39). Although the mechanism of how α-chloralose may affect the DNP-stimulation-evoked bladder reflexes remains unclear, similar reflexes are evoked in conscious, chronic spinal cord injury cats (41), suggesting that α-chloralose anesthesia is not critical to evoking these reflexes.

While the α-antagonist reduced the magnitude of stimulation-evoked and distention-evoked contractions by ∼25%, robust contractions were still evoked by pudendal afferent stimulation, suggesting that the α-adrenergic pathway played only a modulatory role in the stimulation-evoked response. The α-adrenergic innervation of the vesical ganglia is typically described as inhibitory, but there are two distinct actions of α-adrenergic receptors: α₁-receptor-mediated facilitation of ganglionic transmission and α₂-receptor-mediated inhibition of ganglionic transmission (2, 14, 26). Additionally, activation of the bladder neck and proximal urethra is mediated by α₁-receptors (11), and phentolamine decreases bladder neck pressure and suppresses the increase in bladder neck pressure evoked by pudendal afferent stimulation (38).

These findings suggest two possible explanations for the increase in volume thresholds and decrease in contraction magnitude following α-block: loss of facilitation at the vesicle ganglia or loss of activation of the bladder neck. Block of α₂-receptors (by the antagonist atipamezole) decreased bladder excitability (increased bladder capacity and residual urine) in conscious rats (24), suggesting that α₂-receptor block may have contributed to the increase in micturition threshold and decrease in stimulation-evoked contraction magnitudes. The decrease in distention-evoked contraction magnitudes following phentolamine, while not significant, is consistent with phentolamine decreasing bladder excitability and not impairing the pudendal-to-bladder reflex. Regardless of the mechanism, the effects of α-block were not evident following hypogastric transection, providing further evidence that pudendal afferent stimulation-evoked modulation of α-adrenergic bladder activity does not contribute significantly to bladder excitation.

**Hypogastric transection.** Bilateral hypogastric nerve transection was used as an alternate, nonselective means of blocking the sympathetic bladder innervation, and results were similar under surgical and pharmacological block. Hypogastric nerve transection decreased the DTV, consistent with previous findings (31, 35, 50) and also decreased the STV. Additionally, the decrease in volume thresholds was consistent with the volume threshold changes induced by β-block but contrary to the changes due to α-block, suggesting that the β-adrenergic pathway has greater influence over the micturition threshold than the α-adrenergic pathway.

The lack of effect of subsequent administration of adrenergic antagonists on the volume thresholds indicates that hypogastric transection provided functionally complete block of the sympathetic bladder innervation. The pelvic nerve also contains a significant sympathetic component (29), but this may have primarily a vasomotor function (20). The administration of the adrenergic antagonists subsequent to hypogastric nerve transection ensured substantial, if not complete, block of the sympathetic pathways to the bladder, and even under such conditions, robust bladder contractions were still evoked by pudendal afferent stimulation and distension, albeit at smaller bladder volume thresholds. As well, the ability to evoke robust bladder contractions by stimulation of pudendal afferents following hypogastric transection also rules out any role of nonadrenergic hypogastric bladder pathways (9) in the excitatory pudendal-to-bladder reflex.

Low-frequency stimulation of the DNP inhibited distention-evoked reflex contractions of the bladder as previously reported (41, 45). Inhibition of distention-evoked contractions was preserved following hypogastric transection and subsequent adrenergic antagonist administration, and the inhibition was comparable to inhibition evoked prior to hypogastric transection. Previously, it was reported that hypogastric transection reduced bladder inhibition evoked by pudendal nerve stimulation, but inhibition was recovered when the stimulation intensity was increased by factors of two to three (42). In the present study, stimulation intensity was equal to three times the threshold to evoke a reflex EMG response in the external anal sphincter, and the effect of stimulation intensity was not varied. These results suggest that the primary reflex pathways for bladder contraction and inhibition evoked by pudendal afferent stimulation do not engage adrenergic mechanisms.

**Genitovesical reflex.** While previous results suggest a role of the sympathetic bladder pathways in the inhibitory genitovesical reflex (30, 42), our findings suggest that convergence of somatic (pudendal) and parasympathetic (pelvic) afferents is the pathway for reflex bladder excitation by pudendal afferent stimulation. Sympathetic block altered volume thresholds, but it did not abolish the ability to evoke bladder contractions at volume thresholds smaller than those for distention-evoked responses. This suggests that the excitatory genitovesical reflex results from converging spinal inputs from genital and pelvic afferents, providing increased excitatory input to pathways that activate preganglionic parasympathetic bladder efferents. Pelvic and pudendal afferents converge on interneurons in the sacral spinal cord (7, 22), providing a substrate for the excitatory genitovesical reflex. Additionally, the strong correlation between the bladder volume and the magnitude of contractions evoked by DNP stimulation provides evidence that increasing the level of pelvic afferent input drives increased pelvic efferent activity, implying convergence of the pudendal afferent and pelvic afferent inputs in the sacral spinal cord.
That reflex excitation by pudendal afferent stimulation is mediated by the parasympathetic pelvic nerve is supported by the finding that the reflex is abolished by the nicotinic ganglionic blocker hexamethonium bromide. Further, the preservation of reflex bladder activation by pudendal afferent stimulation following acute spinal transection (4–6, 46, 49) and the subsequent abolition of stimulation-evoked contractions by hexamethonium bromide in the spinal transected animal, confirm the spinal origin of the DNP-bladder pathway.

**Conclusion.** The sympathetic bladder pathway does not play a significant role in reflex excitation of the bladder by pudendal afferent stimulation, but sympathetic bladder innervation is important in determining the threshold volumes for stimulation-evoked and distention-evoked reflex bladder contractions. The excitatory pudendal-to-bladder reflex is mediated by increased activation of parasympathetic fibers arising from convergence of pelvic afferent and pudendal afferent fibers in the sacral spinal cord.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

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