Bladder volume-dependent excitatory and inhibitory influence of lumbo-sacral dorsal and ventral roots on bladder activity in rats

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ABSTRACT

This study was undertaken to examine the role of the afferent and efferent pathways of the lumbo-sacral spinal nerve roots in the tonic control of bladder activity. Changes of isovolumetric bladder activity were recorded in 21 sympathectomized female rats under urethane anesthesia following transection of the dorsal (DRT) and ventral (VRT) lumbo-sacral spinal roots, and after intraperitoneal administration of hexamethonium. DRT altered the baseline intravesical pressure in a bladder volume-dependent manner in each animal. The percent change of baseline pressure after VRT following DRT was also dependent upon bladder volume. The percent change of baseline pressure after VRT alone was similarly dependent on bladder volume, but not after VRT followed by DRT. The percent change of baseline intravesical pressure (y)(−9 to +8 cm H₂O, −56 to +46%) after DRT and VRT depended upon bladder volume (x)(y = 44.7 x −40.4) in all rats. Hexamethonium increased the amplitude of small myogenic bladder contractions after DRT and VRT. In conclusion, the bladder is tonically excited or inhibited by a local reflex pathway and by a parasympathetic reflex pathway that depends on connections with the lumbo-sacral spinal cord and the pelvic nerves. Both reflex mechanisms are influenced by bladder volume.
MATERIALS AND METHODS

Adult female Sprague-Dawley rats \( (n = 21) \) weighing 255–350 g were used. The abdomen was opened under urethane anesthesia (0.3 mg/kg intraperitoneally and 0.9 mg/kg subcutaneously), and the hypogastric nerves and sympathetic chains were transected bilaterally near the bifurcation of the abdominal aorta. The ureters were transected and their distal ends were ligated. A transurethral bladder catheter (PE-90, 1.27 mm in outer diameter; Becton Dickinson) was used to record the bladder pressure isovolumetrically with the proximal urethral ligated. The catheter was connected to a 1 mL or 3 mL syringe and a pressure transducer through a three-way stopcock. A chart recorder was used to display bladder pressures. Since the rat bladder is permeable to saline but not to soybean oil (16), the bladder was slowly filled with soybean oil to various volumes (0.25–1.8 mL) that were below or above the threshold in order to evoke isovolumetric reflex contractions. The baseline intravesical pressure was always kept at or below 30 cm H\(_2\)O. Laminctomy (L1–4) was performed to expose the spinal roots. When bladder activity stabilized at 1 h after soybean oil infusion, the amplitude and frequency of spontaneous bladder contractions and the baseline intravesical pressure were measured for 10-min intervals to obtain an estimate of the control of bladder activity.

In order to separately evaluate tonic control of the bladder mediated by pathways in the dorsal and ventral roots, rats were divided into 2 groups. In group 1 \( (n = 11) \), the dorsal roots (L4–S4) were transected first and the ventral roots (L4–S4) were transected later. In group 2 \( (n = 10) \) the sequence was reversed, with the ventral roots being transected first and the dorsal roots transected later. Changes in the amplitude and frequency of isovolumetric spontaneous bladder contractions and the baseline intravesical pressure were also recorded following 1) transection of the dorsal or ventral spinal roots (L4–S4) and 2) administration of hexamethonium bromide (Sigma, St. Louis, USA; 100 mg/kg intraperitoneally), a ganglionic blocking agent, after dorsal and ventral root transection. Transection of the dorsal or ventral roots and administration of hexamethonium were performed at 30-min intervals, and data were obtained for least 10 min after each procedure when the readings were almost stable.

Results are reported as the mean \( \pm \) standard error. Linear regression analysis and Student’s t-test for paired data were used for statistical analysis, and \( p < 0.05 \) was considered to indicate statistical significance.

RESULTS

Effect of initial transection of the dorsal roots on bladder activity

Dorsal root transection (L4–S4) was performed first in 11 rats (group 1). Transection of the dorsal roots significantly decreased the amplitude of spontaneous bladder contractions (from 39.7 \( \pm \) 8.2 cm H\(_2\)O to 4.1 \( \pm \) 1.1 cm H\(_2\)O, \( p < 0.0001 \)) in all animals, and increased the frequency of these contractions (from 0.8 \( \pm \) 0.6/min to 3.4 \( \pm \) 0.6/min, \( p < 0.0001 \)) in 9 animals. In the remaining 2 animals, spontaneous contractions disappeared after dorsal root transection. The bladder volume of these 2 animals was 0.5 mL, while bladder volume ranged between 0.35 and 1.8 mL in the other 9 animals. Baseline intravesical pressure before transection of the dorsal roots ranged between 9 and 29 cm H\(_2\)O (15.5 \( \pm \) 1.8 cm H\(_2\)O). The baseline pressure \( (y) \) was significantly dependent upon bladder volume \( (x) \) in each animal (linear regression; \( y = 8.5 x + 8.3 \), \( r = 0.6089 \), \( p = 0.0481 \)). After transection of the dorsal roots, the baseline pressures changed by \( -4 \) to \( +5 \) cm H\(_2\)O. In rats with a bladder volume equal to or greater than 0.75 mL, the baseline intravesical pressure did not change or increased by 0–5 cm H\(_2\)O increase (0–38%; \( n = 7 \)) after transection of the dorsal roots (Fig. 1A). On the other hand, in rats with a bladder volume equal to or less than 0.65 mL, the baseline intravesical pressure decreased by 1–4 cm H\(_2\)O (11–31%; \( n = 4 \)) after transection of the dorsal roots (Fig. 1B). The percent change of baseline pressure \( (y) \) after dorsal root transection depended significantly upon the bladder volume \( (x) \) in each animal \( (y = 34.4 x - 26.2 \), \( r = 0.6927 \), \( p = 0.0466 \)) (Fig. 2).

Effect of ventral root transection after dorsal root transection on bladder activity

Transection of the ventral roots (L4–S4) at 30 min after dorsal root transection did not influence the amplitude (2.9 \( \pm \) 0.6 cm H\(_2\)O, \( n = 11 \)) and frequency (3.6 \( \pm \) 0.7/min, \( n = 9 \)) of spontaneous bladder contractions. In rats with a bladder volume equal to or greater than 0.75 mL, the baseline pressure did not change or increased by 0–4 cm H\(_2\)O (0–17%; \( n = 6 \)) after transection of the ventral roots (Fig. 1A). On the other hand, in rats with a bladder volume equal to or less than 0.75 mL, baseline pressure decreased by 1–5 cm H\(_2\)O (11–18%; \( n = 5 \)) after transection of the ventral roots (Fig. 1B). The percent change of baseline intravesical pressure \( (y) \) after transection of
Effect of initial transection of the ventral roots on bladder activity

In 10 rats (group 2), ventral root transection (L4–S4) was performed first. Transection of the ventral roots was also significantly dependent upon bladder volume \( (x) \) \( \left( y = 22.4 x - 22.3, \ r = 0.6392, \ p = 0.0297 \right) \) (Fig. 3).
roots significantly decreased the amplitude of spontaneous bladder contractions (from 36.6 ± 8.4 cm H₂O to 1.4 ± 0.3 cm H₂O, p < 0.0001) in all animals and increased the frequency of spontaneous bladder contractions (from 1.1 ± 0.2/min to 3.9 ± 0.2/min, p < 0.0001) in 8 animals. In the remaining 2 animals, spontaneous contractions disappeared after ventral root transection. The bladder volume of these 2 animals was 0.85 mL and 1.0 mL, while the other 8 animals had a bladder volume ranging from 0.4 to 1.75 mL. The baseline intravesical pressure before transection of the ventral roots was 8–28 cm H₂O (18.4 ± 1.9 cm H₂O). Baseline pressure (y) was significantly dependent on bladder volume (x) in each animal (y = 6.9 x + 13.7, r = 0.6615, p = 0.0413). In rats with a bladder volume of 0.65–1.75 mL (n = 5), the baseline pressure decreased by 1–6 cm H₂O (4–25%) after transection of the ventral roots. On the other hand, in rats with a bladder volume of 0.25–0.85 mL (n = 5), baseline pressure decreased by 1–6 cm H₂O (7–37%) after ventral root transection. The percent change of baseline pressure (y) after ventral root transection also depended on the bladder volume (x) in each animal (y = 27.2 x – 24.9, r = 0.7188, p = 0.0228) (Fig. 4).

**Effect of dorsal root transection after ventral root transection on bladder activity**

Transection of the dorsal roots (L4–S4) at 30 min after ventral root transection did not influence the amplitude (1.4 ± 0.4 cm H₂O, n = 10) or frequency (3.8 ± 0.3/min, n = 8) of spontaneous bladder contractions. Changes of the baseline intravesical pressure (−3 to +1 cm H₂O, −30 to +7%) after transection of the dorsal roots were small and were not dependent on bladder volume (Fig. 5).

**Effect of dorsal and ventral root transection on the baseline intravesical pressure**

The percent change of baseline intravesical pressure (y) (−9 to +8 cm H₂O change, −56 to +46% change) after dorsal and ventral root transection compared with before transection in all rats (groups 1 and 2, n = 21) was significantly dependent on bladder volume (x) (y = 44.7 x − 40.4, r = 0.7591, p < 0.0001) (Fig. 6).

**Effect of hexamethonium on bladder activity after dorsal and ventral root transection**

Administration of hexamethonium to 18 rats (groups 1 and 2) after transection of the dorsal and ventral roots significantly increased the amplitude of spontaneous bladder contractions (from 2.6 ± 0.3 cm H₂O to 3.6 ± 0.8 cm H₂O, p < 0.0001) (Fig. 7). The frequency of spontaneous contractions (from 4.2 ± 0.2/min to 4.6 ± 0.6/min) and the baseline intravesical pressure (17.2 ± 2.2 cm H₂O to 15.5 ± 2.1 cm H₂O) were not influenced by administration of hexamethonium. Changes of the amplitude, frequency, and baseline pressure did not depend upon bladder volume.

**DISCUSSION**

The present results demonstrate that the parasympathetic preganglionic pathways in the pelvic nerves tonically regulate bladder activity with or without bladder afferents to the spinal cord and that a hexamethonium-sensitive peripheral mechanism also tonically regulates bladder activity, increasing or decreasing bladder tone depending upon the bladder volume.

In this study, the sympathetic pathways in the hy-
pogastric nerves and sympathetic chains were transected bilaterally at the level of the bifurcation of the abdominal aorta in all rats. After transection of the L4–S3 dorsal and ventral roots, which include all afferent and efferent fibers from the pelvic nerves (12), the bladder was decentralized. In this state, administration of the ganglionic blocking agent hexamethonium resulted in an increase in the amplitude of small spontaneous bladder contractions. This effect of hexamethonium suggested the existence of a local reflex pathway (5, 6) which might be composed of bladder afferents and their collaterals projecting to the pelvic ganglia and postganglionic neurons projecting back to the bladder via the pelvic nerves. This finding is consistent with the results of our previous in vivo study (17) using a neonatal rat spinal cord-bladder preparation with transection of the lumbosacral ventral roots, in which electrical stimulation of the lumbosacral afferent roots inhibited spontaneous bladder contraction induced by bladder distension. This inhibition was blocked by administration of hexamethonium, suggesting that afferents activate a peripheral cholinergic inhibitory mechanism. However, since the effects of hexamethonium were not dependent on bladder volume in this study, it is possible that the local reflex pathway does not depend on mechano-sensitive afferent activity induced by bladder distension. Seki et al. (13) reported that detrusor smooth muscle cell membranes showed spontaneous electrical activity in a study of normal guinea pig muscle strips. Therefore, the increase in the amplitude of small spontaneous bladder contractions after administration of hexamethonium may mean that desynchronized intrinsic contractile activity of bladder smooth muscle cells was released from the control of inhibitory local reflexes after treatment by hexamethonium (6).

Dorsal root transection before ventral root transection altered the baseline intravesical pressure in a manner that depended on bladder volume. Ventral root transection before or after dorsal root transection also changed the baseline pressure depending on the bladder volume. However, dorsal root transection after ventral root transection did not influence bladder activity. These results demonstrate that the peripheral efferents of sensory nerves detected in the in vitro neonatal rat spinal cord-bladder preparation (17) are not active in adult rats under anesthesia with urethane, and that parasympathetic preganglionic neurons provide tonic output with or without bladder afferent input to the spinal cord.

When bladder volume was small, transection of the ventral roots after dorsal root transection decreased the baseline pressure. This result implies
that inhibitory postganglionic neurons are tonically inhibited, or excitatory postganglionic neurons are tonically facilitated, by afferent collaterals and preganglionic neurons. On the other hand, when the bladder volume was large, transection of the ventral roots after dorsal root transection increased the baseline pressure. This result indicates that inhibitory postganglionic neurons were tonically facilitated, or excitatory postganglionic neurons were tonically inhibited, by afferent collaterals and preganglionic neurons under our experimental conditions. After transection of dorsal and ventral roots, postganglionic neurons receive input from only afferent collaterals. Under these conditions the input to the local efferent neurons may be only partially dependent upon bladder volume. Possible transmitters in the afferent collaterals are acetylcholine, substance P, calcitonin gene-related peptide (CGRP), nitric oxide, pituitary adenylate cyclase-activating polypeptide (PACAP), or adenosine triphosphate (ATP), which could activate hexamethonium-sensitive and -resistant receptors (1, 6, 7, 9, 11).

When the ventral roots were still intact after dorsal root transection, postganglionic neurons received input from afferent collaterals and preganglionic neurons with activity that was constant and not dependent upon bladder volume. In this state, our results indicate that the parasympathetic system has both excitatory and inhibitory effects on bladder activity depending on the bladder volume. One efferent pathway having two opposing volume-dependent influences on bladder activity may mean that multiple neurotransmitters (inhibitory and excitatory) are released from the same neurons and that the release of these transmitters is differentially regulated by the level of activity in the two input pathways and is possibly dependent on bladder volume. For example, after dorsal root transection if postganglionic neurons receive a small input from afferent collaterals and a constant input from preganglionic neurons, the total input to postganglionic neurons would not induce bladder volume-dependent excitatory or inhibitory effects. However, ventral root transection after dorsal root transection did actually induce a volume-dependent change of bladder activity, so the constant preganglionic neuronal output may amplify the volume-dependent input from afferent collaterals (Fig. 8). In this state, amplified excitatory or inhibitory inputs to postganglionic neurons may be able to induce excitation or inhibition of these neurons depending on the input volume. During the micturition reflex, large-amplitude bladder contractions occur and there is massive firing of preganglionic axons in the pelvic nerves (2), so the small reflex mechanisms identified by the present study may have a minimal effect on bladder activity. In the future, frequency-dependent release of neurotransmitters from postganglionic neurons will need to be investigated.

As mentioned above, preganglionic axons in the pelvic nerves arising in the ventral lumbosacral roots have both excitatory and inhibitory tonic influence on bladder tone in rats. These effects increase or decrease baseline pressure by several cm H2O. The excitatory responses are active at small bladder volumes and the inhibitory responses are active at large bladder volume. The volume at which the response shifts from excitation to inhibition was 0.9 mL (y = 44.7 x − 40.4, when y = 0, x = 0.9), which is the same as micturition volume (0.9 mL) in awake rats (8) and near threshold volume (0.6 mL) that evokes reflex micturition in urethane-anesthetized rats (18). Therefore, the parasympathetic pathway may act in an excitatory or inhibitory manner, respectively, when the bladder volume is below or above the threshold volume that evokes reflex micturition. The inhibitory efferent element of this pathway is speculated to promote urine storage by keeping the baseline intravesical pressure low. On the other hand, the excitatory effect of this pathway on bladder tone seems to be less important, except for reflex contractions induced via this pathway. However, it is possible that the excitatory effect also promotes urine storage by closing the bladder neck and ureteral orifices, although it is possible that the increase of baseline pressure in a bladder volume-dependent manner was induced by increased ATP release from the bladder epithelium (also in a bladder volume-dependent manner) (4).

In conclusion, bladder tone in rats after sympathectomy is tonically controlled by local excitatory or inhibitory reflex pathways that employ efferent and afferent axons in the pelvic nerves and circuitry in the spinal cord and in the pelvic ganglia. These tonic excitatory or inhibitory efferent effects of the pelvic nerves are dependent on bladder volume. The volume at which the tonic influence of the pelvic nerves on the bladder changes from excitation to inhibition is 0.9 mL, which is equal to the reported threshold volume that evokes the micturition reflex in awake rats (8). Therefore, the parasympathetic pathway is not only important for micturition, but also for urine storage.
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REFERENCES
