Analysis of the afferent limb of the vesicovascular reflex using neurotoxins, resiniferatoxin and capsaicin

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Received 25 September 2000; accepted in final form 13 June 2001.

Chuang, Yao-Chi, Matthew O. Fraser, Yongbei Yu, Jonathan M. Beckel, Satoshi Seki, Yasukazu Nakanishi, Hitoshi Yokoyama, Michael B. Chancellor, Naoki Yoshimura, and William C. de Groat. Analysis of the afferent limb of the vesicovascular reflex using neurotoxins, resiniferatoxin and capsaicin. Am J Physiol Regulatory Integrative Comp Physiol 281: R1302–R1310, 2001.—The afferent limb of the vesicovascular reflex (VV-R) evoked by distension or contraction of the urinary bladder (UB) was studied in urethane-anesthetized female rats by examining the changes in VV-R after administration of C-fiber afferent neurotoxins [capsaicin and resiniferatoxin (RTX)]. Systemic arterial blood pressure increased parallel (5.1 to 53.7 mmHg) with graded increases in UB pressure (20 to 80 cmH2O) or during UB contractions. The arterial pressor response to UB distension was significantly reduced (60–85%) by acute or chronic (4 days earlier) intravesical administration of RTX (100–1,000 nM) or by capsaicin (125 mg/kg sc) pretreatment (4 days earlier). Chronic neurotoxin treatments also increased the volume threshold (>100%) for eliciting micturition in anesthetized rats but did not change voiding pressure. Acute RTX treatment (10–50 nM) did not alter the arterial pressor response during reflex UB contractions, whereas higher concentrations of RTX (100–1,000 nM) blocked reflex bladder contractions. It is concluded that VV-R is triggered primarily by distension- and contraction-sensitive C-fiber afferents located, respectively, near the luminal surface and deeper in the muscle layers of the bladder.

PATIENTS WITH SPINAL CORD INJURY above the T6 segment are prone to hypertensive episodes, which result from spinally mediated reflexes that increase sympathetic nerve activity (5–7, 9, 21, 26). The condition, known as autonomic dysreflexia, is most commonly elicited by bladder manipulation, distension, or contraction. The increased arterial pressure during autonomic dysreflexia may cause retinal, cerebral, and subarachnoid hemorrhage and death (25). Although the mechanisms underlying the emergence of autonomic dysreflexia are uncertain, it has been hypothesized that the disorder may occur due to damage of bulbo-spinal pathways and elimination of baroreceptor control of spinal sympathetic reflex mechanisms (12, 21). Alternatively, autonomic dysreflexia may arise as a result of a reorganization of spinal autonomic reflex pathways, formation of new synaptic connections, and expression of new receptors or upregulation of normal receptors after degeneration of bulbo-spinal and propriospinal connections after spinal cord injury (12, 18, 20).

Traditionally, autonomic dysreflexia has been treated with vasodilators, α-adrenergic blocking agents, or calcium channel blockers (25). In addition, recently it was reported that intravesical administration of afferent neurotoxins, capsaicin or resiniferatoxin (RTX), reduced autonomic dysreflexia as well as detrusor hyperreflexia in spinal cord-injured patients (2, 9). These observations suggest that capsaicin-sensitive C-fiber afferent nerves in the bladder (2) may be involved in autonomic dysreflexia.

Previous experiments in anesthetized spinal cord-intact rats showed that systemic administration of capsaicin reduced the arterial pressor response accompanying isovolumetric contractions of the bladder [i.e., the vesicovascular reflex (VV-R); Ref. 3]. Osborn et al. (23) used one-step bladder distension from 0 to 20 mmHg in spinal cord-injured rats and found the VV-R was completely blocked by intravesical lidocaine or autonomic ganglionic blockade. Thus the VV-R, which has been detected in various species (rats, cats, and dogs; Refs. 1, 3, 5, 7) under anesthesia, may be a useful model to study autonomic dysreflexia.

The present study was undertaken to examine the effects of bladder distension and bladder contractions on arterial pressor responses in adult spinal-intact rats. We hypothesize that different afferent pathways (i.e., volume receptors and tension receptors) might be involved in VV-R evoked by bladder distension and bladder contraction, and these two pathways might have different sensitivity to intravesical RTX. RTX, a C-fiber afferent neurotoxin with a 1,000-fold greater...
potency than capsaicin (2), was instilled into bladder to analyze the contribution of Aδ- and C-fiber afferents to arterial pressor responses. Systemic capsaicin pretreatment 4 days before the experiment was used to evaluate the role of capsaicin-resistant afferents in the pressor responses.

METHODS

Surgical procedures. Forty adult female Sprague-Dawley rats (250–300 g) were anesthetized with urethane (1.2 g/kg sc). Body temperature was maintained in the physiological range using a heating lamp. Systemic arterial pressure was recorded via a pressure transducer connected to a cannula in the common carotid artery. PE-50 tubing (Clay-Adams, Parsippany, NJ) was inserted into the bladder through the urethra and tied in place by a ligature around the urethral orifice. A ventral laparotomy was performed, and both ureters were transected and tied distally. The wound was left open to expose the bladder and allow confirmation of complete bladder emptying. All protocols involving the use of animals in this study were approved by the Animal Care and Use Committee of the University of Pittsburgh School of Medicine.

Cystometrogram. The PE-50 tubing in the urethra was connected via a three-way stopcock to a pressure transducer and to a syringe pump for recording intravesical pressure and for infusing saline into the bladder, respectively. After the bladder was emptied, a cystometrogram (CMG) was performed by slowly filling the bladder (0.04 ml/min) with saline. The infusion pump was turned off with the onset of rhythmic bladder contractions, and the bladder was then maintained under constant volume conditions. The volume of saline sufficient to induce bladder contractions was defined as the micturition volume threshold. The number and amplitude of reflex isovolumetric bladder contractions (>15 cmH2O) were measured during a 15-min period of bladder filling with saline or RTX at different concentrations.

Micturition pattern. To evaluate the impact of RTX on micturition, we placed some conscious rats in metabolic cages (Nalgene metabolic cage, Nalgene-NUNC, Rochester, NY) and measured voided urine for a 24-h period 1 day before as well as 1 and 4 days after the intravesical administration of RTX. Urine was collected in a cup on a Grass force-displacement transducer, which permitted measurements of micturition frequency and volume (10).

Bladder distension. Intravesical pressure was increased in a stepwise manner (20–80 cmH2O with 10-cmH2O increment frequency and volume (10)).

Arterial pressor responses were also elicited by bladder distension at intravesical pressures above a threshold pressure of 20 cmH2O. The arterial pressure responses increased in a graded manner with increasing distension pressures and reached a plateau (mean 37.6 mmHg increase in systolic pressure; range 29.4–51.5 mmHg) at intravesical pressures between 60 and 70 cmH2O (Fig. 1B). The pressor responses were significantly reduced (mean 8.5 mmHg, 77.4% decrease) after capsaicin pretreatment as evidenced by a rightward shift in the VV-R vs. intravesical pressure curve (Figs. 1D, 2, A and C).

Acute effect of RTX (10–100 nM). Before application of RTX into the bladder, the reproducibility of CMGs and VV-R after serial distensions of the bladder was examined in three rats. After four consecutive series of 30-min bladder distensions with a volume of saline equivalent to the micturition volume threshold, CMG parameters and VV-R induced by bladder contraction or distension were not significantly changed (Table 2).
indicating that repeated bladder distensions had no significant effects on bladder activity and VV-R.

Instillation of 10 nM RTX into bladder for 30 min also did not significantly change the contraction number and amplitude of isovolumetric bladder contractions (Table 2). However, administration of increasing concentrations of RTX in a cumulative manner from 10 to 100 nM completely blocked the micturition reflex in

Table 1. Effect of capsaicin (125 mg/ml sc) and RTX (intravesical) administration 4 days before the experiment on micturition and vesicovascular reflexes

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Capsaicin (n = 7)</th>
<th>100 nM RTX (n = 5)</th>
<th>500 nM RTX (n = 5)</th>
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<tr>
<td>BCA, cmH₂O</td>
<td>37.9 ± 4.8</td>
<td>32.7 ± 3.6</td>
<td>32.3 ± 1.9</td>
<td>35.8 ± 5.3</td>
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<tr>
<td>Basal SBP, mmHg</td>
<td>119.9 ± 3.9</td>
<td>120.1 ± 2.9</td>
<td>117.8 ± 3.8</td>
<td>116.0 ± 3.0</td>
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<td>Change in SBP during bladder contraction, mmHg</td>
<td>14.5 ± 2.1</td>
<td>5.4 ± 0.8**</td>
<td>5.4 ± 1.0*</td>
<td>2.2 ± 1.1†</td>
</tr>
<tr>
<td>Micturition volume threshold, ml</td>
<td>0.40 ± 0.10</td>
<td>0.94 ± 0.08*</td>
<td>1.1 ± 0.1*</td>
<td>1.0 ± 0.2*</td>
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<tr>
<td>Maximal change in SBP during bladder distension, 70 cmH₂O</td>
<td>37.6 ± 7.6</td>
<td>8.5 ± 0.8**</td>
<td>12.4 ± 3.5*</td>
<td>9.0 ± 0.9*</td>
</tr>
<tr>
<td>Contraction number in 15 min after first contraction</td>
<td>6.8 ± 0.9</td>
<td>5.1 ± 1.1</td>
<td>4.0 ± 0.9</td>
<td>2.2 ± 1.1†</td>
</tr>
</tbody>
</table>

Values are means ± SE. BCA, bladder contraction amplitude; SBP, systolic blood pressure. **P < 0.05, significant difference from control; †P < 0.05, significant difference from 100 nM resiniferatoxin (RTX)-pretreated group.
six of nine animals after the highest concentration. The arterial pressor responses accompanying reflex bladder contractions were not significantly changed by RTX treatment before the block of micturition at the highest RTX concentration (Table 2).

**Acute effect of RTX (100, 500, 1,000 nM).** As shown in Fig. 3, a CMG revealed that acute administration of 100 nM RTX significantly increased in the number of isovolumetric bladder contractions from 4.3 ± 0.8 to 10.9 ± 1.8 in 15 min (P < 0.05), whereas the contraction amplitude was not significantly changed (Table 3). However, 500 and 1,000 nM RTX blocked the micturition reflex 10–20 min after drug administration (Fig. 3). Before the isovolumetric contractions were completely abolished, the arterial pressor responses accompanying reflex bladder contractions were not significantly changed during RTX treatment. However, the pressor responses triggered by bladder distension were significantly reduced by RTX treatment in a concentration-dependent manner (Figs. 2 and 4). Single treatment with RTX in concentrations of 500 or 1,000 nM (n = 3, respectively) had the same effect on the micturition reflex and pressor responses as cumulative treatment in concentrations of 100, 500, and 1,000 nM.

After pretreatment with capsaicin, the intravesical administration of 100 nM RTX still activated bladder activity and increased contraction number (from 5.1 ± 1.1 to 8.3 ± 1.5, P < 0.05), but it did not significantly change the arterial pressor responses. Higher concentrations of RTX (500 and 1,000 nM) significantly reduced the remaining pressor response. *P < 0.05, **P < 0.01, ***P < 0.001, compared with control (t-test).

**Table 2. Effect of acute intravesical administration of RTX on micturition and vesicovascular reflex induced by isovolumetric bladder contractions**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 9)</th>
<th>Saline (n = 3)</th>
<th>10 nM (n = 9)</th>
<th>25 nM (n = 9)</th>
<th>50 nM (n = 9)</th>
<th>100 nM (n = 9)</th>
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</thead>
<tbody>
<tr>
<td><strong>BCA, cmH₂O</strong></td>
<td>37.9 ± 4.8</td>
<td>36.3 ± 4.5</td>
<td>39.4 ± 6.7</td>
<td>38.8 ± 6.7</td>
<td>47.2 ± 8.5</td>
<td>40.5 ± 7.3</td>
</tr>
<tr>
<td><strong>Change in SBP</strong></td>
<td>14.5 ± 2.1</td>
<td>21.3 ± 2.8</td>
<td>19.0 ± 2.9</td>
<td>21.2 ± 3.2</td>
<td>22.6 ± 5.2</td>
<td>16.7 ± 2.9</td>
</tr>
<tr>
<td><strong>Contraction number in 15 min after first contraction</strong></td>
<td>6.8 ± 0.9</td>
<td>7.2 ± 0.4</td>
<td>7.9 ± 1.4</td>
<td>7.0 ± 1.7</td>
<td>7.4 ± 1.2</td>
<td>3.6 ± 0.8*</td>
</tr>
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</table>

Values are means ± SE. Values for saline were obtained after 4 consecutive series of 30-min bladder distensions with a volume of saline equivalent to the micturition volume threshold. *P < 0.05, significant difference from control.

_AJP-Regulatory Integrative Comp Physiol_ • _VOL 281_ • _OCTOBER 2001_ • www.ajpregu.org
posttreatment, respectively. The mean micturition frequency in the 100 nM RTX-treated group was 23.4 ± 0.9, 27.6 ± 2.5, and 30.6 ± 4.6 for 24 h for control, day 1, and day 4 posttreatment, respectively. The mean micturition frequency in the 500 nM RTX-treated group was 18.2 ± 1.3, 21.0 ± 5.4, and 19.8 ± 1.6 for 24 h for control, day 1, and day 4 posttreatment, respectively.

Four days after treatment with 100 nM RTX, CMGs performed under urethane anesthesia revealed that the mean contraction number (4.3 ± 0.8 vs. 4.0 ± 0.9 per 15 min) and contraction amplitude were not significantly changed (37.9 ± 4.8 vs. 32.3 ± 1.7 cmH2O); however, micturition volume threshold was significantly increased (0.4 ± 0.1 vs. 1.1 ± 0.1 ml; Fig. 5A and Table 1). In animals pretreated with 500 nM RTX, the contraction number was significantly decreased (4.0 ± 0.9 vs. 2.0 ± 0.7) and micturition volume threshold was significantly increased (0.4 ± 0.1 vs. 1.0 ± 0.2 ml); however, the contraction amplitude was not significantly changed (37.9 ± 4.8 vs. 35.8 ± 5.3 cmH2O; Table 1). Mean systolic arterial pressure was 116.2 ± 6.8 and 112.3 ± 3.5 mmHg in 100 and 500 nM RTX-pretreated groups, which was similar to the control group. However, arterial pressure responses during bladder contractions were significantly reduced in the 100 nM RTX (5.4 ± 1.0 mmHg, 63% decrease) and 500 nM RTX (2.2 ± 1.1 mmHg, 85% decrease)-pretreated groups compared with the untreated group (14.5 ± 2.1 mmHg). The mean maximal arterial pressor responses to bladder distension (70 cmH2O) were also significantly reduced (12.4 ± 3.5 and 9.0 ± 0.9 mmHg in the 100 and 500 nM RTX-pretreated animals, respectively) compared with the untreated group (Fig. 5B and Table 1).

**DISCUSSION**

The present study revealed that acute or chronic (4 days) intravesical treatment with RTX (100, 500, and 1,000 nM) reduced the arterial pressor responses (VV-R) triggered by bladder distension. However, arterial pressor responses during bladder contractions were reduced by 4-day pretreatment, but not by acute treatment. Capsaicin pretreatment 4 days before the experiment in a dose that is known to desensitize C-fiber bladder afferents reduced the VV-R during bladder contractions or distension but did not alter the acute effect of RTX (100 nM) to facilitate the micturition reflex. We propose that acute intravesical RTX treatment desensitizes mucosal C fibers that mediate the pressor responses triggered by bladder distension but does not have an immediate effect on C fibers located deeper in the muscle layers of the bladder wall that mediate pressor responses during bladder contractions (Fig. 6). An effect of RTX on the latter afferents had a more delayed onset and was noted 4 days after RTX treatment. This might be due to differences in the concentration of RTX in different regions of the bladders (i.e., high in the mucosa, lower in the muscle) as a result of incomplete penetration of the bladder wall (16). Afferents exposed to a lower concentration of RTX might require a longer time to degenerate or desensitize.

In animal and human studies, reflex increases in arterial pressure that are elicited by bladder filling coincide with increased sympathetic nerve activity (5, 6, 7, 26). After sympathectomy or blockade of vesical afferent activity in animal studies, the arterial pressor responses are abolished (23, 26). These observations suggest that the responses are mediated by activation of bladder afferents, which reflexly stimulate sympathetic nerve outflow and cause vasoconstriction or car-
diac stimulation (13). Because the VV-R can be elicited in anesthetized normal as well as spinal cord-injured animals (3, 12, 23), it is possible to use the former as a model to study the mechanisms that mediate autonomic dysreflexia.

The present results indicate that different afferent pathways are involved in the VV-R induced by bladder distension or bladder contractions. The VV-R induced by distension was reduced by acute or chronic intravesical RTX (100–1,000 nM) instillation; however, the VV-R induced during isovolumetric contractions was only reduced by chronic treatment. One possible explanation for this difference is that the rate of penetration of RTX through the urothelium is slow and that only afferent nerves within or immediately beneath the urothelium are acutely desensitized (Ref. 8). The presence of C-fiber afferent volume receptors in the mucosa of the rat bladder is consistent with this speculation (22). Volume receptors are activated by bladder distension but do not fire in response to bladder contractions. On the other hand, tension receptors located in the muscle layers deeper in the bladder wall respond to isovolumetric bladder contractions as well as distension. These afferent nerves would be less accessible to intravesically administered RTX and therefore would be expected to desensitize more slowly of RTX through the urothelium.

### Table 3

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>100 nM</th>
<th>500 nM</th>
<th>1000 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BCA, cmH_2O</strong></td>
<td>60.6 ± 7.5</td>
<td>54.9 ± 7.5</td>
<td>50.8 ± 8.4</td>
<td>46.8 ± 7.2*</td>
</tr>
<tr>
<td><strong>Change in SBP during bladder contraction, mmHg</strong></td>
<td>20.8 ± 3.8</td>
<td>21.2 ± 4.3</td>
<td>17.0 ± 2.1</td>
<td>11.4 ± 2.6*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05, significant difference from control.

![Fig. 4](http://ajpregu.physiology.org/) Acute effect of intravesical administration of RTX on vesicovascular reflex induced by isovolumetric bladder contractions. Top tracings in each record are blood pressure (mmHg) and bottom tracings are bladder pressure (cmH_2O). Acute RTX administration (control, A; 100 nM, B; 500 nM, C; and 1,000 nM, D) decreased the blood pressure response in a concentration-dependent manner.
than mucosal afferents. Thus the differential sensitivity of distension- and contraction-evoked VV-R to intravesical RTX is most reasonably attributed to different locations of two types of mechanosensitive afferents in the bladder wall. A substantial proportion of both types of afferents must be sensitive to vanilloid receptor agonists because systemic capsaicin, which would have equal access to both types of afferents or chronic RTX, significantly reduced contraction-evoked as well as distension-evoked VV-R. However, systemic capsaicin or chronic RTX did not completely block VV-R, indicating that capsaicin-resistant presumably A-fiber afferents also contribute to VV-R (Fig. 6).

It is noteworthy that chronic intravesical RTX treatment did not influence voiding function in conscious rats housed in metabolism cages. Voiding frequency and the volume of urine per void were not altered, indicating that afferent input from the bladder that triggers the sensation of bladder fullness and initiates voluntary voiding was not compromised by intravesical RTX administration. However, when the same rats were studied under urethane anesthesia, the bladder capacity measured during a slow infusion CMG was significantly increased (163% increase). Systemic capsaicin treatment produced a similar increase in bladder capacity in urethane-anesthetized rats (24, 28), indicating that activity in C-fiber bladder afferents modulates but is not essential for reflex voiding. Other investigators (10) have also reported that voiding in conscious rats is not suppressed by intravesical administration of RTX (100 nM). This supports the idea proposed previously by Maggi and Meli (15) that different types of bladder afferents trigger voluntary and reflex voiding in the rat.

Intravesical RTX also elicited acute excitatory and depressant effects on bladder activity in urethane-anesthetized rats. As noted by other investigators (9), RTX elicited an initial increase in the frequency of reflex bladder contractions. This effect has also been noted after topical application of capsaicin to the bladder and has been attributed to direct stimulation of C-fiber afferents (17). Chronic (4–6 days) intravesical treatment with RTX prevents this acute excitatory effect of RTX (10). However, in the present study, systemic capsaicin pretreatment 4 days before the experiment with a large dose that would be expected to completely desensitize C-fiber bladder afferents reduced but did not completely eliminate the acute excitatory effect of RTX. This raises the possibility that moderate concentrations of RTX administered intravesically also stimulate A-fiber afferents. Patch-clamp studies (27) showed that a small percentage of bladder A-fiber afferent neurons is responsive to capsaicin. This type of bladder afferent is essential for triggering the micturition reflex in the rat (19). Thus direct excitation of A fibers in the bladder would be expected to induce bladder hyperactivity.

Administration of increasing concentrations of RTX to a maximum of 100 nM blocked the micturition reflex in ~70% of animals, and higher concentrations (500–1,000 nM) blocked reflex bladder activity in all animals. This effect is very likely due to a nonspecific
depression of A-fiber afferents as noted previously after the acute systemic administration of high doses of capsaicin (3). This depressant effect of vanilloids on A fibers is of relatively short duration (12–14 h) compared with the prolonged desensitization of C fibers that can persist for days to weeks (3).

It is noteworthy that intravesical application of 100 nM RTX produced a prominent stimulating effect on bladder activity when administered to an otherwise untreated bladder, whereas administration of increasing concentrations of RTX to a maximum of 100 nM blocked the micturition reflex. This discrepancy in the effect of 100 nM RTX is likely due to nonspecific desensitization of A-fiber afferents by the smaller concentrations administered before 100 nM RTX.

In conclusion, VV-R can be mediated by Aβ- and C-fiber mechanosensitive bladder afferents; however, C fibers play a major role. Intravesical RTX treatment desensitizes C fibers and reduces the VV-R but preserves micturition function. Intravesical RTX administration could be an alternative treatment for autonomic dysreflexia.

**Perspectives**

The urinary bladder is innervated by several types of afferent nerves (Aβ-fiber myelinated and C fiber unmyelinated) that subserve multiple functions. Some afferents induce bladder sensations (e.g., fullness and pain) that initiate voluntary voiding, and others seem to trigger involuntary voiding reflexes. The present results indicate that the rat bladder contains at least two populations of C-fiber afferents that have either different sensitivities or different access to intravesically administered RTX. One population, which appears to be responsive to volume changes, mediates VV-R evoked by bladder distension, whereas the other, which appears to be responsive to changes in bladder wall tension, mediates VV-R evoked by bladder contraction and/or distension. The sensitivity of the former VV-R to acute intravesical administration of RTX provides indirect support for the proposal of Morison (22) that C-fiber afferent volume receptors are located near the epithelial surface of the bladder. The two populations of C-fiber afferents do not contribute to voluntary voiding but have an important role in modulating reflex voiding under anesthesia. This finding emphasizes the importance of C-fiber afferents as targets for therapy in patients with detrusor hyperactivity, involuntary voiding, and incontinence and raises the possibility that appropriate doses of afferent neuropeptides could depress abnormal bladder function without affecting normal voluntary voiding. However, the present experiments also demonstrated that high concentrations of RTX administered intravesically can completely block reflex bladder contractions, presumably by depressing Aβ-afferent pathways. This finding raises a caution about high-dose RTX therapy that could produce short periods of complete urinary retention.

The authors thank the Afferon Company for supplying RTX.

This work was supported by Grants from National Institute of Diabetes and Digestive and Kidney Diseases (DK-49430 and -57267) and Spinal Cord Research Foundation, Paralyzed Veterans of America (#1862).

**REFERENCES**


