Abdominal muscle activity during voiding in female rats with normal or irritated bladder

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Departments of 1Pharmacology and 3Urology, Faculty of Medicine, Dalhousie University, Halifax, Nova Scotia, Canada; and 2Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, Mexico

Submitted 28 July 2005; accepted in final form 20 December 2005

**Cruz, Yolanda, and John W. Downie.** Abdominal muscle activity during voiding in female rats with normal or irritated bladder. *Am J Physiol Regul Integr Comp Physiol* 290: R1436–R1445, 2006. First published December 22, 2005; doi:10.1152/ajpregu.00556.2005.—The aims of the present study were to determine in female rats whether abdominal muscle discharges during normal voiding and to describe the effect of bladder irritation on this visceromotor activity. The sensory pathway of this reflex was also determined. Electromyograms (EMGs) indicated that in awake rats, the abdominal muscle was consistently activated during spontaneous voiding and during voiding induced by saline infusion. Similarly, in anesthetized animals, the muscle discharged during urine expulsion. The abdominal EMG activity was not abolished by hypogastric (Hgnx) or sensory pudendal neurectomy (SPDnx). SPDnx dramatically decreased the intercontraction interval and voided volume. Acetic acid infusion reduced the intercontraction interval and increased bladder contraction duration. It also reduced the pressure threshold for evoking the abdominal EMG response and increased the EMG duration and amplitude. Although SPDnx and Hgnx modified some urodynamic parameters, they did not reverse the acetic acid effect on EMG activity. Thus the afferents activating the visceromotor reflex during normal voiding and the increased reflex in response to acetic acid are probably both carried by the pelvic nerve. Abdominal muscle activity induced by bladder distension has been considered to be a pain marker. However, we conclude that in female rats, the abdominal muscle is reflexively activated during physiological urine expulsion. On the other hand, bladder irritation is marked by an exaggeration of this abdominal visceromotor reflex.

**METHODS**

In total, 42 adult female (253–308 g) Wistar rats were used in this study. The rats were maintained on a 12:12-h light-dark cycle with food (rodent pellets) and water provided ad libitum. The experimental protocol was approved by the Dalhousie University Committee on Laboratory Animals, according to the guidelines of the Canadian Council on Animal Care and the American Physiological Society’s “Guiding Principles in the Care and Use of Animals.”

Abdominal muscle activity in unanesthetized rats. Electromyographic activity (EMG) of the external oblique muscle was recorded in the lateral abdominal region (Fig. 1, site b) and urine was collected during spontaneous (n = 3) or induced (n = 3) micturition. A muscle was considered to have been activated when the amplitude of the EMG trace increased by at least 30% above the basal level associated with no body movement (quiet standing). For the week before testing, the rats were handled and placed in an acrylic restraint tube for 10 min per day. During testing, the behavior of the rats was observed and marked on the EMG record.

One hour before spontaneous micturition was recorded, non-bladder-cannulated rats were anesthetized with isoflurane and the EMG electrodes were positioned. EMGs were recorded through a pair of Teflon-coated silver wires (uncoated diameter 0.05 mm, bared for −2 mm at the tip). The coated portion of the recording wires was saturated to the skin. The bared tips were inserted into the abdominal muscle through a small skin incision that was then closed. Isoflurane was discontinued, and 5 ml of saline solution were injected subcutane-

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ABDOMINAL MUSCLE ACTIVITY DURING VOIDING IN FEMALE RATS

clitoris, and the rostral portion of the left pubic bone was removed to insert wires in the EUS (Fig. 1, site d). The skin was sutured closed to prevent drying of the tissue. Bladder pressure and EMG signals (band pass 300 Hz–3 kHz) were amplified and digitized at 200 Hz and 5 kHz, respectively (Power1401; Cambridge Electronic Design). In 14 rats, CMGs and EMGs of the three abdominal muscles were recorded simultaneously and urodynamic and EMG parameters were analyzed as described previously (14). In three rats, the saline infusion was continued for 2 h.

In 10 rats, either the hypogastric nerve (Hgn, n = 5) or the sensory pudendal nerve (SPdn, n = 5) was localized according to previous studies (3, 13, 38), and a silk loop was placed around it. CMGs and EMG activity were assessed during saline infusion before and 15 min after transection of either nerve (Hgnx or SPdnx). In some rats, continuous infusion was interrupted, the bladder was drained through the implanted cannula, and a single fill to voiding was carried out. Voided volume and residual volume in the bladder after voiding were measured. Voiding efficiency (in %) was calculated as [voided volume/(voided volume + residual)] × 100%.

In 12 other rats, saline was infused for 20 min, and then the infusion was changed to 0.2% acetic acid for a further 1 h. In eight rats, prepared as described above, Hgnx or SPdnx were performed before (Hgnx, n = 2; SPdnx, n = 2) or after (Hgnx, n = 2; SPdnx, n = 2) acetic acid infusion. CMGs and EMGs were compared among these conditions.

Statistical analysis. Values in text are presented as means ± SE. GB-STAT statistical software (version 5.0; Dynamic Microsystems) was used for analysis. Urodynamic parameters were analyzed using ANOVA followed by Dunnett’s test for post hoc comparisons. The data measured before and after bladder inflammation were compared using paired Student’s t-test. P < 0.05 was taken to indicate a significant difference.

RESULTS

Abdominal EMG activity in unanesthetized rats. At least two spontaneous urine expulsions occurred during the 90-min observation period with ICI and voided volume averaging 41 ± 1.46 min and 1.0 ± 0.08 ml, respectively (n = 3). The behaviors recorded during the test were as follows: quiet (no body movements), postural changes (movements of limbs or head), sniffing (movement of nose and whiskers with audible breathing sound), defecation, and urine voiding. The rats were mostly quiet with some postural changes and sniffing. Two rats defecated 2–5 min after they were introduced into the tube. The external oblique muscle EMG showed only baseline activity and no discharge when the rats were quiet. However, other behaviors were associated with low-amplitude EMG activity. Rats were usually quiet when voiding, but the external oblique muscle consistently showed high-amplitude EMG discharge during each urine expulsion (Fig. 2A).

In the saline infusion group (n = 3), the above behaviors and EMG activity were observed in the first 5 min. However, after that period, the rat was quiet for long periods with only basal EMG activity. Voiding occurred approximately every 10 min, and voided volume was slightly smaller than in spontaneous micturition episodes (Table 1).

Most bladder contractions were single peaks without HFOs. The external oblique muscle showed a large-amplitude EMG discharge during each bladder contraction (Fig. 2B). The abdominal EMG activity started before urine expulsion, at a bladder pressure between the micturition threshold and the peak voiding pressure (~10 mmHg, Table 1). In 90% of the bladder contractions recorded, urine started to be expelled at

OUSLY. THE RAT WAS PLACED FOR 90 MIN IN A SEMICYLINDRICAL ACRYLIC RESTRAINING TUBE (6.5 CM WIDE × 4.5 CM HIGH × 10 CM LONG) THAT HAD A HOLE IN THE FLOOR FOR URINE COLLECTION. VOIDED FLUID WAS COLLECTED IN A SMALL CONTAINER THAT WAS CONTINUOUSLY WEIGHED USING A SMALL POLYETHYLENE BEAKER ON A STRAIN GAUGE. EMG SIGNALS (BAND PASS 300 Hz–3 kHz) WERE AMPLIFIED AND DIGITIZED AT 200 Hz. THE WEIGHT OF VOIDED FLUID WAS DIGITIZED AT 100 Hz AND RECORDED ALONG WITH EMG. THE DATA WERE DISPLAYED AND STORED ON A PENTIUM 4 COMPUTER RUNNING SPIKE 2 SOFTWARE (VERSION 5.01; CAMBRIDGE ELECTRONIC DESIGN).

FOR INDUCED MICTURITION, THE RATS WERE ANESTHETIZED WITH ISOFLURANE, THE BLADDER WAS EXPOSED BY A MIDLINE ABDOMINAL INCISION, AND A SILASTIC CANNULA (1.5-MM OUTER DIAMETER) WAS SECURED IN THE BLADDER DOME. THE CANNULA WAS TUNNELED SUBCUTANEOUSLY AND ATTACHED TO A PEDESTAL THAT WAS THEN FIXED ON THE SKULL WITH SCREWS AND DENTAL ACRYLIC. THE ABDOMINAL INCISION WAS CLOSED IN TWO LAYERS. ANTIBIOTIC (BAYTRIL, 2.5 mg/kg) AND ANALESIC (BUPRENORPHINE, 0.03 mg/kg) WERE INJECTED AFTER THE SURGERY. ON POSTCANNULATION DAYS 7–10 AND 1 h BEFORE THE EXPERIMENT, THE ANIMALS WERE ANESTHETIZED WITH ISOFLURANE AND EMG ELECTRODES WERE POSITIONED AS DESCRIBED ABOVE. THE ANIMALS WERE PLACED IN THE ACRYLIC RESTRAINT TUBE. SALINE WAS INFUSED THROUGH THE CANNULA USING A SYRINGE INFUSION PUMP (0.1 ml/min), AND BLADDER PRESSURE WAS MONITORED ON A SIDE LINE. THE PRESSURE-VOLUME RELATIONSHIP (CYSTOMETROGRAM, CMG), EMG, AND URINE WEIGHT WERE RECORDED SIMULTANEOUSLY. EMG AND VOIDED URINE WEIGHT WERE RECORDED AS DESCRIBED FOR SPONTANEOUS MICTURITION. AFTER 20 min OF CONTINUOUS SALINE INFUSION, THE NEXT THREE BLADDER CONTRACTIONS AND EMGS WERE ANALYZED, AND THE FOLLOWING PARAMETERS WERE DETERMINED (SEE FIGS. 4 AND 5): INTERCONTRACTION INTERVAL (ICI), PRESSURE THRESHOLD, MAXIMUM PRESSURE OF THE BLADDER CONTRACTION, CONTRACTION DURATION, NUMBER OF HIGH-FREQUENCY BLADDER PRESSURE OSCILLATIONS (HFOs), AND VOIDED VOLUME (14).

ABDOMINAL EMG ACTIVITY IN ANESTHETIZED RATS WITH NORMAL OR IRRITATED BLADDER. STUDIES WERE CARRIED OUT IN 36 FEMALE RATS ANESTHETIZED WITH URETHANE (1.2 g/kg ip). RATS WERE PLACED ON A HEATED PAD, AND THE BLADDER WAS CANNULATED AS DESCRIBED ABOVE. A SKIN INCISION WAS MADE ON THE RIGHT SIDE OF THE ABDOMINAL WALL, AND EMG RECORDING WIRES WERE INSERTED INTO THE EXTERNAL OBLIQUE, INTERNAL OBLIQUE, OR RECTUS ABDOMINIS MUSCLE. THE FIRST TWO WERE RECORDED IN THE LATERAL AND INGUINAL REGIONS (FIG. 1, SITES B AND C), AND RECTUS ABDOMINIS MUSCLE WAS RECORDED IN THE MIDLINE REGION (FIG. 1, SITE A). IN SOME RATS, ANOTHER INCISION WAS MADE AT THE PERINEAL SKIN LATERAL TO THE BLADDER.
bladder pressures of 10 ± 0.7 mmHg and finished before the bladder pressure reached maximum pressure (Fig. 3). Urine expulsion duration was 2.6 ± 0.14 s, much shorter than the overall contraction duration.

Abdominal EMG activity in anesthetized rats. CMGs and abdominal muscle EMGs were simultaneously recorded during saline infusion. In CMGs, the bladder pressure trace during micturition had two peaks with HFOs between the peaks (Figs. 4 and 5).

In the overall group of 36 rats, 32 (89%) showed a consistent relationship between the abdominal muscle EMG discharge and the bladder contraction (Figs. 4 and 6). In the remaining four rats, no EMG activity was recorded in any muscle during bladder contractions.

The external oblique muscle EMG activity threshold and duration were −15 mmHg and 4 s, respectively (intact group, Table 2). The muscle started to discharge before bladder contraction reached the first micturition peak, continued during HFOs, and finished as pressure began to fall after the second micturition peak (Fig. 5). Each EMG record had at least two components characterized by amplitude, with the component of higher amplitude discharging during HFOs (Fig. 5). In some animals (n = 4), the EUS was recorded along with the external oblique muscle. In contrast to the abdominal muscle, the EUS discharged in a burst pattern during HFOs and continued as a tonic activity after the bladder contraction had finished (Fig. 5).

The EMG activity pattern of the internal oblique muscle was the same as that of the external oblique muscle (Fig. 6). However, the EMG activity of the rectus abdominis muscle was not consistent during voiding. It did not discharge in five rats. In seven others, the EMG was present only during some bladder contractions (Fig. 6).

When saline infusion was continued for 2 h (n = 3), no significant differences were found in the external oblique muscle EMG duration and amplitude between the first three and last three bladder contractions (duration: 3.8 ± 0.8 vs. 3.5 ± 0.5 s; amplitude: 1.0 ± 0.3 vs. 0.9 ± 0.5 V).

Table 1. Urodynamic and external oblique muscle EMG parameters of induced micturition in unanesthetized rats

<table>
<thead>
<tr>
<th>ICI, s</th>
<th>PT, mmHg</th>
<th>MP, mmHg</th>
<th>CD, s</th>
<th>EMGT, mmHg</th>
<th>EMGD, s</th>
<th>VV, ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>502 ± 749</td>
<td>4.5 ± 0.62</td>
<td>19 ± 3.0</td>
<td>18 ± 2.3</td>
<td>9.6 ± 2.33</td>
<td>4.4 ± 0.12</td>
<td>0.87 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 3). ICI, intercontraction interval; PT, pressure threshold; MP, maximum pressure; CD, contraction duration; EMGT, EMG threshold; EMGD, EMG duration; VV, voided volume.
In 10 other rats, CMGs and EMGs were recorded 15 min after Hgnx (n = 5) or SPdnx (n = 5). Compared with intact animals (Table 2), Hgnx increased pressure threshold and decreased voided volume but altered no other characteristics of voiding. In contrast, SPdnx produced dramatic changes in the urodynamic pattern. SPdnx significantly reduced ICI, maximum pressure, contraction duration, and voided volume and eliminated HFOs (Table 2). It also converted the usual double-peaked bladder contractions into single peaks (similar to Fig. 8D). Neither Hgnx nor SPdnx abolished the EMG activity of the external or internal oblique muscles. Although the EMG duration was slightly decreased in neurectomized animals, the differences from control were not statistically significant in either case (Table 2). Voiding efficiency was determined before and after SPdnx in four rats. Efficiency fell significantly after SPdnx (intact: 60.8 ± 2.5%; SPdnx: 21.8 ± 2.1%).

Abdominal EMG activity in anesthetized rats with irritated bladder. CMGs and EMGs of the external oblique, internal oblique, and rectus abdominis muscles were recorded in 12 female rats before and after bladder inflammation induced by acetic acid. In five of the rats, EUS was also recorded.

During saline infusion, the external and internal oblique muscle EMG activity discharged consistently during each bladder contraction in 10 of the 12 rats. Rectus abdominis muscle was activated in only four rats. In two rats, none of the abdominal muscles responded during bladder contractions induced by saline infusion.

Acetic acid caused profound changes in both the voiding pattern and the abdominal EMG activity. After 30 min of acetic acid infusion, ICI, pressure threshold, and voided volume were reduced significantly. Contraction duration was increased (Table 3). Maximum pressure was clearly increased in five rats, but on average it was not statistically significant. Acetic acid also significantly increased the EMG duration and amplitude of the external oblique muscle and reduced the threshold pressure for onset of discharge (Table 3).

These abdominal muscle changes were clearly present after 15 min of acetic acid (Fig. 7B) and reached a steady level after 30 min of continuous infusion (Fig. 7C). The duration of the discharge was clearly related to the bladder contraction duration. Thus longer bladder contraction activates the abdominal muscle for a longer period.

<table>
<thead>
<tr>
<th>Group</th>
<th>ICI, s</th>
<th>PT, mmHg</th>
<th>MP, mmHg</th>
<th>CD, s</th>
<th>VV, ml</th>
<th>EMGT, mmHg</th>
<th>EMGD, s</th>
</tr>
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<tbody>
<tr>
<td>Intact (n = 10)</td>
<td>165 ± 22.3</td>
<td>3.6 ± 0.6</td>
<td>19.6 ± 1.6</td>
<td>13.8 ± 0.7</td>
<td>0.3 ± 0.02</td>
<td>14.6 ± 1.4</td>
<td>4.0 ± 0.6</td>
</tr>
<tr>
<td>Hgnx (n = 5)</td>
<td>138 ± 22.2</td>
<td>5.9 ± 0.85*</td>
<td>15.7 ± 1.4</td>
<td>13.2 ± 0.4</td>
<td>0.18 ± 0.03*</td>
<td>17.0 ± 1.1</td>
<td>2.9 ± 0.14</td>
</tr>
<tr>
<td>SPdnx (n = 5)</td>
<td>78 ± 8*</td>
<td>2.8 ± 0.35</td>
<td>12.1 ± 1.0†</td>
<td>10.4 ± 1.1*</td>
<td>0.16 ± 0.03†</td>
<td>13.5 ± 2.0</td>
<td>2.7 ± 0.44</td>
</tr>
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</table>

Values are means ± SE. *P < 0.05; †P < 0.01 vs. control.
EUS activity did not show a strong response to acetic acid (Fig. 7). In three of the five rats after 15 min of acetic acid infusion, EUS EMG duration increased by 15–20 s. In general, after 30 min of acetic acid infusion, the EUS tonic activity after infusion, EUS EMG duration increased by 15–20 s. In general, all three abdominal muscles consistently discharged during each bladder contraction in the 12 rats studied.

In 4 of the 10 rats, the effect of acetic acid on bladder and abdominal muscle activity seemed to be stronger. Bladder contraction and EMG duration lasted up to 48 and 55 s, respectively (see example in Fig. 8B). After 25 min of acetic acid infusion, the basal EMG activity was also increased. The effects on bladder contraction duration and abdominal EMG activity of the 45 min of continuous acetic acid infusion were reversed upon resumption of saline infusion for 20 min (Fig. 8D).

Although neurectomies influenced some CMG parameters, the increased activity of the abdominal muscle induced by acetic acid was not abolished by Spdnx (n = 2, Fig. 8) or Hgnx (n = 2, Fig. 9). Previous Spdnx (n = 2) or Hgnx (n = 2) also did not prevent acetic acid infusion-induced EMG changes (data not shown). Because the direction of effects in these neurectomy groups was the same regardless of treatment order, the data were combined for the following analysis (Spdnx, n = 4; Hgnx, n = 4). The irritative effects of acetic acid on some CMG parameters appeared to be accentuated by Spdnx (cf. Table 3). ICI (intact saline: 170 ± 16 s; Spdnx acetic acid: 70 ± 9.8 s) and threshold pressure (intact saline: 4.1 ± 0.08 mmHg; Spdnx acetic acid: 1.7 ± 0.2 mmHg) were significantly reduced, and contraction duration (intact saline: 13.6 ± 0.77 s; Spdnx acetic acid: 18 ± 2.3 s) was significantly increased. On the other hand, maximum pressure was not significantly affected. EMG parameters also were shifted toward greater sensitivity and increased response rather than being obtunded. Threshold for EMG discharge was reduced (intact saline: 16 ± 1.6 mmHg; Spdnx: 10 ± 1.9 mmHg), and EMG amplitude (intact saline: 1.0 ± 0.2 V; Spdnx acetic acid: 1.9 ± 0.4 V) and duration (intact saline: 3.6 ± 0.3 s; Spdnx acetic acid: 10 ± 2.6 s) were significantly increased. The magnitude of these effects implies that they were due primarily to the presence of acetic acid (cf. Table 3) and that Spdnx had little influence on the changes. Saline infusion reversed the effect of the acetic acid on both bladder contraction and abdominal EMG activity (Fig. 8D). However, the resulting bladder contraction characteristics were more similar to those during saline infusion after Spdnx (single peak, shorter duration, reduced amplitude) than to those in intact rats.

Overall, Hgnx in the presence of acetic acid left CMG parameters similar to the preirritation, preneurectomy values. There was no significant effect on ICI, maximum pressure, or contraction duration, but the pressure threshold for micturition was reduced (intact saline: 4.4 ± 1.0 mmHg; Hgnx acetic acid: 2.0 ± 0.6 mmHg). EMG parameters were shifted toward greater sensitivity and increased response rather than being obtunded. The threshold for EMG discharge was reduced (intact saline: 15.4 ± 1.5 mmHg; Hgnx acetic acid: 10 ± 0.9 mmHg), and EMG amplitude (intact saline: 0.8 ± 0.1 V; Hgnx acetic acid: 1.5 ± 0.15 V) and duration (intact saline: 3.2 ± 0.5 s; Hgnx acetic acid: 5.8 ± 0.7 s) were significantly increased. However, it is likely that the EMG changes reflect an effect of acetic acid unaltered by Hgnx (cf. Table 3).

Table 3. Urodynamic and external oblique muscle EMG parameters in the same female rats with normal or irritated bladder

<table>
<thead>
<tr>
<th>Condition</th>
<th>ICI, s</th>
<th>PT, mmHg</th>
<th>MP, mmHg</th>
<th>CD, s</th>
<th>VV, ml</th>
<th>EMG, mmHg</th>
<th>EMGD, s</th>
<th>EMGA, V</th>
</tr>
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<tbody>
<tr>
<td>Normal</td>
<td>178±7.6</td>
<td>3.7±0.42</td>
<td>19±0.81</td>
<td>15.2±0.66</td>
<td>0.32±0.018</td>
<td>16.2±1.1</td>
<td>3.5±0.36</td>
<td>1.0±0.5</td>
</tr>
<tr>
<td>Irritated</td>
<td>111±7.9†</td>
<td>2.8±0.23†</td>
<td>20±1.2</td>
<td>19±1.2†</td>
<td>0.18±0.022‡</td>
<td>10±0.8‡</td>
<td>7.1±1.16‡</td>
<td>1.6±0.7*</td>
</tr>
</tbody>
</table>

Values are means ± SE (n = 12 for CMG parameters, n = 10 for EMG parameters). "Irritated" values were determined 30 min after initiation of 0.2% acetic acid infusion. *P < 0.05; †P < 0.01; ‡P < 0.001 vs. normal (paired t-test). EMGA, EMG amplitude.
DISCUSSION

The present study showed for the first time that in female rats, abdominal muscles are reflexively activated during normal voiding and that this abdominal visceromotor reflex is exaggerated under conditions of bladder irritation. We also found that the sensory pudendal nerve regulates female micturition and that its transection dramatically decreases voided volume and increases voiding frequency.

Fig. 7. EMG activity of the EO and EUS muscles during bladder contractions elicited in the same anesthetized female rat by continuous saline infusion (A and D) or by 15 (B and E) or 30 min of 0.2% acetic acid (C and F) infusion (0.1 ml/min). Larger and longer EO EMG activity was elicited in the irritated urinary bladder condition (B, C, E, and F). D–F show the pericontraction records for records in A–C at expanded time scales. Voltage and pressure scales refer to all panels. Time scale refers only to A–C.

Fig. 8. EMG activity of EO muscle during bladder contractions elicited in the same female rat by continuous saline infusion (A and D) or acetic acid infusion (0.1 ml/min) (B and C) before (A and B) and after (C and D) bilateral transection of the sensory branch of the pudendal nerve. Compared with saline infusion (A), contraction duration and EMG activity during bladder contraction and during bladder filling increased dramatically after 25 min of acetic acid infusion (B). After neurectomy, the effect of acetic acid on the EMG persisted, but bladder contraction duration was slightly decreased (C). Subsequent return to saline infusion in the neurectomized rat reversed the effect of acetic acid on both bladder contraction and EMG activity (D). Note that after neurectomy (D), bladder contractions during saline infusion are single peaks with duration and amplitude reduced from the intact condition (A). Time, pressure, and voltage scales apply to all panels.
Abdominal visceral motor reflex during normal voiding. Abdominal muscles participate in several physiological processes such as sneezing, coughing, defecation, and parturition (24). They can be activated voluntarily or through somatosomatic (postural changes) or visceral somatic reflexes (sneezing, coughing, defecation, and parturition). The present study showed that abdominal muscles are also activated during normal voiding in female rats. This abdominal muscle activation would be expected to raise intra-abdominal pressure, and an increase in intra-abdominal pressure and postural changes have been observed in awake rats at the time of voiding (Andersson KE, personal communication). In this circumstance, the abdominal activity may be interpreted as straining (i.e., a voluntary response) or may be part of a voiding-related posture. However, the abdominal visceral motor reflex activation also was associated with voiding in anesthetized female rats, implying that it did not result from postural changes or voluntary straining.

EMG duration was similar in awake and anesthetized rats, but the pressure threshold to induce EMG discharge was slightly lower in unanesthetized rats. With respect to the urodynamic parameters, ICI, pressure threshold, and contraction duration were higher in awake than in anesthetized rats. These differences probably reflect a greater bladder capacity in the unanesthetized condition, and decreased voiding efficiency in anesthetized rats has been reported previously (37, 45, 71, 72). In support of this conclusion, we found that voided volume was more than twice as great in awake rats than in anesthetized rats.

HFOs are a common feature of voiding in anesthetized rats, where they occur between two bladder pressure peaks (14, 34, 60), and urine flows for the short time during these pressure oscillations (60). We did not record EUS EMG activity in unanesthetized rats, but the bladder pressure response during voiding was different from that seen in anesthetized rats. Bladder contractions in our awake rats were characterized by a single peak, with urine flow occurring simultaneously with external oblique muscle discharge and before the maximum intravesical pressure was reached. A similar observation, of a single bladder pressure peak with no HFOs, was made in a previous study of awake female rats (67). The lack of HFOs on the bladder pressure trace of awake rats is puzzling, because HFOs have been considered to be required for efficient urine expulsion (12). However, HFOs are small in female rats, and block of EUS activity and generation of HFOs more dramatically affects voiding efficiency in males than in females (14). Possibly the lack of detection of HFOs is a technical problem. In the awake condition, the effect of the thin EUS on bladder pressure may be masked by tonic detrusor pressure or by an abdominal muscle contribution to an intra-vesical pressure increase. These differences in urodynamic parameters may be attributed to inhibitory effects of anesthetics on the micturition reflex (37, 40, 45). The fact that the bladder pressure peak occurs after the completion of urine expulsion in awake rats implies that bladder smooth muscle is still contracting while the urethra closes. Bladder smooth muscle contractions are long-lasting (4–9 s) (22). Furthermore, an abrupt onset and tonic discharge of the EUS after voiding closes the bladder outlet and raises bladder pressure (14). Thus urine expulsion time is dependent on the urethra opening period and not on bladder contraction time. We found previously that EUS contraction did not completely occlude the urethra in anesthetized female rats (14). Again, anesthesia may contribute to this discrepancy.

Micturition is a complex spinal-bulbospinal reflex. In rats, somatic, sympathetic, and parasympathetic motor neurons of the lower urinary tract are located at lumbosacral spinal segments (46). Sensory information from the lower urinary tract is carried by hypogastric, pelvic, and the sensory branch of the pudendal nerve (1, 46). The sensory pudendal nerve carries afferents from the urethra but not from the bladder (1, 35, 68). Motor innervation of the lower urinary tract arrives through pelvic, hypogastric, and pudendal nerves (46).

In our neurectomy study, Hgnx slightly decreased ICI and increased pressure threshold. The urodynamic changes seen after Hgnx are consistent with elimination of efferent sympathetic outflow and agree with the idea that the hypogastric nerve mainly modulates urinary continence through an action on the bladder (1, 65). The small voided volume observed after the neurectomy could be a result of the shorter period of filling due to decreased bladder accommodation.

The dramatic effect of SPdnx on urodynamic pattern indicates that in the female rat, the sensory pudendal nerve, also called the clitorial nerve, regulates normal voiding. In female rats, this nerve innervates the clitoris and clitoral sheath, and in male rats, the penile shaft and glans penis (13, 38, 50). It has not been described as innervating the urethra in rats, but in men it innervates the penile urethra. The sensory pudendal nerve has been related primarily to sexual reflexes (39, 51, 54, 63, 69), but clearly it influences micturition as well (20, 58).
fact that this nerve regulates both sexual and micturition processes may explain the association between sexual and voiding dysfunctions (55, 64). The physiological mechanism underlying the urodynamic changes produced by SPdnx is not clear, but both afferent and efferent urethral pathways appear likely to be involved. We found that SPdnx decreased significantly ICI, voided volume, maximum pressure, and contraction duration. Similar results were reported in female rats after bilateral denervation of the EUS (14). Because voiding efficiency is also reduced by SPdnx, we suspect that the urodynamic changes are due to impaired emptying that lowers voided volume and results in increased voiding frequency during continuous infusion. The issue is then, is driving force compromised, or is outflow impaired?

Bladder contraction may be compromised because maximum pressure is reduced. However, because the pelvic nerves are intact, it seems unlikely that this deficiency is caused by direct impairment of parasympathetic drive to the detrusor muscle. A positive feedback loop, in which activation of urethral afferents in the pudendal nerve enhances bladder contraction, has been demonstrated in various species (2, 9, 19, 20, 25, 53, 57). Thus SPdnx may have reduced driving force by interrupting a urethral afferent pathway, contributing to facilitation of parasympathetic excitatory drive to the bladder.

In rats, EUS bursting is an important factor contributing to voiding efficiency (14, 32). However, the urodynamic changes observed in the present study are not likely related to a deficient bursting of the striated sphincter activity, because the neurectomy was specific to the sensory branch of the pudendal nerve and the motor branch was left intact. On the other hand, it has been proposed that during voiding, the main role of urethral sympathetic innervation is to relax the smooth muscle to open the urethra for urine release (27, 73). In rats, the sensory branch of the pudendal nerve carries sympathetic efferent axons innervating the urethral smooth muscle, and electrical nerve stimulation induces urethral relaxation (29).

Thus our neurectomy also may have impaired outflow by interrupting a urethral sympathetic efferent pathway in the SPdn that is important for urethral smooth muscle relaxation and lowered resistance to flow. Further studies are needed to determine the physiological mechanisms underlying the regulatory role of the SPdn in urethrovessical function.

Because neither Hgxn nor SPdnx eliminates the abdominal visceromotor reflex, we conclude that the afferent pathway of this reflex travels through the pelvic nerve. The finding that electrical stimulation of the proximal portion of a cut pelvic nerve induced abdominal muscle activity in rats and cats (15, 18) supports this suggestion.

The role of abdominal visceromotor activity in voiding is uncertain. Respiratory frequency has been reported to increase during bladder contractions in awake rats (59). The abdominal activity during bladder contraction may support this change in breathing or may increase intra-abdominal pressure to facilitate voiding. In human beings, it has been suggested that a certain level of abdominal strain to increase the intra-abdominal pressure is necessary to activate the levator ani muscle. This muscle is attached to the urethra, and its contraction opens the urethra, facilitating urine expulsion (56, 57).

**Abdominal visceromotor reflex during voiding in rats with irritated bladder.** Our study confirmed previous findings that bladder irritation induced by acetic acid increased voiding frequency and reduced voided volume in unanesthetized and anesthetized rats (26, 41). This phenomenon may result from sensitization of bladder C fibers (6, 17, 41) and increased activity of spinal and supraspinal neurons related to bladder function (4, 5, 7, 26, 41, 42). Antagonists of tachykinins, N-methyl-D-aspartate, and nitric oxide synthase partially reverse the acetic acid-induced increase in c-fos and the reduction in ICI, implying that the corresponding transmitters may be involved in the acetic acid facilitation of the micturition reflex (26, 28, 41).

Another possible explanation of decreased ICI and voided volume could be increased urethral resistance resulting from increased periurethral striated activity during voiding. Acetic acid increased the EUS and external anal sphincter activity during voiding (26, 62). Although in our data the EUS did not show dramatic changes during acetic acid infusion, the tonic activity seemed to be more robust than during saline infusion. On the other hand, the rhythmic activity, necessary for urine expulsion, was decreased. More studies are necessary to determine the relative contributions of decreases in bladder capacity or voiding efficiency to the acetic acid-induced dysfunction.

In halothane-anesthetized rats or isoflurane-anesthetized mice, the abdominal muscle responds to noxious visceral stimulation but not to bladder distension in the normal physiological range (48, 49). Bladder inflammation lowered the abdominal visceromotor reflex threshold in awake mice and augmented the muscle response (49). Thus the abdominal visceromotor reflex induced by urinary bladder distension has been considered to be a pain marker (48, 49). However, we have shown that the reflex is present during physiological bladder distension and voiding in both unanesthetized and urethane-anesthetized rats. This difference in results may be related to the anesthetic. Inhalation anesthetics increase γ-aminobutyric acid and glycine receptor activity by ∼100%, compared with ∼30% for urethane, and thus they could increase spinal inhibitory controls on micturition (21, 23, 30, 43, 44). Although inhalation anesthetics greatly depress the micturition reflex (37), the depression is not specific for micturition, because the abdominal visceromotor reflex normally induced by gentle touching of the cervix in urethane-anesthetized rats also was abolished in isoflurane-anesthetized rats (Cruz Y, unpublished observation). Perhaps the afferents inducing the abdominal activity in response to normal visceral stimulation are more susceptible to anesthetic block than are the nociceptors.

Our data imply that the abdominal visceromotor reflex during voiding is increased in response to acetic acid and that the exaggerated response mainly depends on bladder afferents traveling in the pelvic nerve. Because the urethra is also stimulated during acetic acid expulsion and the pelvic nerve also innervates the urethra, we cannot reject the possibility that some abdominal muscle responses result from urethral stimulation.

Acetic acid decreased EMG threshold and increased the amplitude of the abdominal muscle EMG. Decreased EMG threshold implies that sensitization of C fiber afferents could be involved. Increased EMG amplitude raises the possibility that bladder irritation also may activate silent C fibers that activate other abdominal motor units. Silent fibers could activate motoneurons not activated during voiding induced by saline infusion, such as the rectus abdominis muscle.
Both 1% and 0.1% acetic acid are reported to cause severe mucosal degeneration, submucosal edema, infiltration of inflammatory cells, and disruption of the urothelial layer (4, 41). Normalization of bladder function after such a severe damage would be expected to require repair of the damage. In our study, the effect of 45–60 min of 0.2% acetic acid infusion on bladder and abdominal visceromotor reflex was reversed to almost normal after 20 min of saline infusion, in agreement with a previous study (62). It is likely that longer exposure to acetic aid could produce bladder inflammation and more persistent changes in micturition reflexes (41). The effects of acetic acid observed in our study are consistent with a direct, reversible activation of TRPV1 receptors or acid-sensing ion channels rather than by inducing inflammation (11, 31).

**Perspectives**

The role of abdominal muscles in the micturition of human beings has been controversial for several years (8, 33, 52, 57, 70). Abdominal straining during voiding has been considered to reflect urethral dysfunction. However, it also has been suggested that abdominal muscle activation during normal voiding is necessary to activate pelvic striated muscles, whose contraction opens the urethra (57). In addition, increased intra-abdominal pressure by abdominal wall contraction may help to empty the urinary bladder efficiently. We have presented data showing that the abdominal visceromotor reflex is activated during normal voiding in female rats and that its activity is exaggerated when the bladder is irritated. This raises the question of whether the presence of visceromotor reflexes is a good indicator of nociception as well as whether they play a role in determining voiding efficiency in the awake condition.

**ACKNOWLEDGMENTS**

We thank Leslie Ingram for technical assistance.

**GRANTS**

This work was supported by the Canadian Institutes for Health Research (MOP-42443). Y. Cruz received financial support from National Institute of Neurological Disorders and Stroke Contract N01 NS-2-2342 and the Reynolds Postdoctoral Fellowship in Biomedical Research.

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ABDOMINAL MUSCLE ACTIVITY DURING VOIDING IN FEMALE RATS