Vesicoanal, urethral, and urethrovaginal reflexes initiated by lower urinary tract irritation in the rat

KARL B. THOR,1,2 AND MARK A. MUHLHAUSER3

1GenuPro, Incorporated, Research Triangle Park, Morrisville 27560; 2Division of Urology, Department of Surgery, Laboratory of NeuroUrology, Duke University Medical Center, Durham Veterans Administration Medical Center, Durham, North Carolina 27705; and 3Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285

Thor, Karl B., and Mark A. Muhlhauser. Vesicoanal, urethroanal, and urethrovaginal reflexes initiated by lower urinary tract irritation in the rat. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1002–R1012, 1999.—Irritation of the urinary bladder causes activation of normally “silent” nociceptive primary afferent fibers. In the present study, it is reported that irritation of the urinary bladder or urethra with infusion of 0.5% acetic acid robustly activates motoneurons that innervate the striated muscle of the external anal sphincter via spinal reflex mechanisms. The activation of anal motoneurons following irritation of the bladder and urethra are termed vesicoanal and urethral reflexes, respectively. The reflexes can be mimicked by acute application of capsaicin to the bladder and urethra, and they show desensitization following prolonged topical application of capsaicin or following chronic systemic pretreatment with capsaicin. The reflexes can be demonstrated in chronic spinal cord-transected animals, indicating that the reflex pathways are organized within the spinal cord. The urethral urethral reflex is also physiologically activated by urethral distension and increases in intraluminal pressure. In addition to activation of anal sphincter activity, slight distension, pressure increases, or instillation of 0.5% acetic acid into the urethra inhibited bladder contractions through activation of an inhibitory urethrovaginal reflex. These reflexes are discussed in terms of clinical characteristics of urethritis and prostatitis. Anecdotally, it was discovered that the bladder can buffer acetic acid.

micturition; bladder; nociception; anal sphincter; urethra

UNDER NORMAL CONDITIONS, unmyelinated afferent fibers (i.e., C fibers) in the lower urinary tract do not respond to distension of the bladder (4, 23). However, electrophysiological recordings of bladder afferent fibers showed that following irritation or inflammation of the bladder this normally silent subset of unmyelinated afferent nerve fibers are activated by bladder distension (13, 14). It is thought that activation of silent nociceptors may produce clinical signs of abdominal pain, dysuria, urinary urgency, and incontinence (16, 18).

Although study of the peripheral changes in bladder nociceptors is of great value, it is also important to evaluate central nervous system components of lower urinary tract irritation. Previous studies in animals have shown that bladder irritation can activate central nervous system mechanisms. For example, topical application of capsaicin, the pungent ingredient of peppers, can induce a single, brief activation of supraspinal micturition reflex pathways (22). Additionally, instillation of acetic acid into the bladder can cause c-fos mRNA expression in spinal cord neurons and increases in bladder activity (2, 3). However, there appear to be no animal studies of the effects of bladder irritation on the striated anal or urethral sphincters despite clinical indications that bladder hyperreflexia induces activation of these and other pelvic floor muscles (1, 6, 26).

In the present study, central reflex responses to lower urinary tract irritation were explored to allow a separation of the afferent and efferent components of bladder irritation in an in vivo preparation (i.e., discovery of a response that was driven by bladder afferent fibers but was not dependent on bladder efferent fibers). On the basis of 1) the close anatomical proximity within the sacral spinal cord between the central processes of bladder afferent fibers and the dendrites and cell bodies of external urethral and external anal sphincter motoneurons and 2) physiological evidence that bladder, colon, urethral, and anal sphincter functions are often coordinated (9–11), the effects of lower urinary tract irritation on urethral and anal sphincter activity were examined.

Briefly, it was found that infusion of a dilute acetic acid solution into the lower urinary tract robustly activated anal sphincter striated muscles. The organization of this activity (i.e., through a spinally or supraspinally organized reflex), its dependence on capsaicin-sensitive afferent neurons, and the relative contribution from bladder or urethral afferent fibers were explored. It was also discovered that manipulation of the urethra produced marked inhibition of urinary bladder activity. A previous study had shown that capsaicin injection into the urethra inhibits bladder activity (8). The present study expands on those findings by exploring the effects of urethral stimuli that more closely approximate naturally occurring pathophysiological conditions (e.g., distension, pressure, and infusion of a weak acid). Preliminary findings were previously published in abstract form (24).

MATERIALS AND METHODS

General preparation and transvesical infusion. Female Sprague-Dawley (n = 53) and Wistar (n = 4) rats (200–300 g) were anesthetized with 1.4 g/kg urethan (administered as divided doses of 0.7 g/kg ip and 0.7 g/kg sc), which was supplemented with isoflurane during the surgical procedures. The urinary bladder was exposed via abdominal incision and...
cannulated with PE-60 tubing attached to a 22-gauge needle that was inserted through the dome of the bladder. A purse string suture (5–0), tied around the bladder distal to a PE-60 "cuff" on the needle, held the tubing in place and prevented damage of the bladder by the needle tip. The cannula was then connected to an infusion pump (Harvard Apparatus, South Natick, MA) and a pressure transducer (Viggo Spectramed, Oxnard, CA), via a three-way connector, to allow bladder filling (0.04–0.1 ml/min) with 0.9% saline or 0.5% acetic acid and pressure measurement, respectively. The abdominal incision was then covered with plastic wrap. A small incision was made in the perineum to allow placement of electromyographic (EMG) electrodes into the periurethral musculature. EMG electrodes were implanted in the muscular tissue of the external anal sphincter as well. EMG electrodes were connected to an AC preamplifier (model PS511, Grass, Quincy, MA) with low- and high-pass filters set at 10 and 3,000 Hz, respectively.

Arterial pressure was monitored via a catheter in the carotid artery, which was connected to a Gould-Statham pressure transducer. EMG potentials, ratemeter output, bladder and arterial pressure measurements, heart rate, and expired CO₂ measurements (Sensor Medics, Anaheim, CA) were recorded on a physiograph (model TA-4,000, Gould) and stored on an eight-channel digital audio tape recorder (model PC-108M, Sony, Tokyo, Japan).

The total number of EMG spikes associated with each bladder contraction was counted using a ratemeter/spike discriminator (RAD III, Winston Electronics, Millbrae, CA). The amplitude and duration parameters for each counted spike were set to eliminate smooth muscle potentials (i.e., small, long-duration potentials). This number was then divided by the duration of the anal sphincter firing associated with each bladder contraction to obtain a value for EMG in spikes per second. Duration of anal sphincter firing was defined as the period of time in which there was an obvious uninterrupted increase of EMG activity associated with a micturition contraction (compare with Fig. 1C), expressed in seconds. In those cases in which there was no obvious association between bladder activity and anal sphincter activity during saline infusion, the number of spikes that occurred during the bladder contraction served as control. Data are expressed as means ± SE. A paired t-test, assuming unequal variances, was used to determine significance at the P = 0.05 level.

Topical capsaicin. In seven animals, the cannulated bladder was withdrawn from the abdominal cavity and a vehicle-soaked (10% ethanol, 10% Tween 80 in saline) cotton ball was applied topically to the bladder for 20 min. The vehicle-soaked cotton ball was then replaced with a capsaicin-soaked (1 mg/ml dissolved in 10% ethanol, 10% Tween 80 in saline) cotton ball. Plastic wrap was positioned under the bladder to prevent contact of capsaicin with surrounding tissue. After treatment, the bladder was rinsed with saline and returned to the abdominal cavity.

Capsaicin pretreatment. Six Sprague-Dawley and four Wistar rats were anesthetized with isoflurane and then subcutaneously injected with capsaicin (50–120 mg/kg sc in 10% ethanol, 10% Tween 80 in saline; Sigma). Animals were maintained under light isoflurane anesthesia for an additional 1.5 h. Eight Sprague-Dawley rats were treated as such but were injected only with the capsaicin vehicle. Animals were returned to their cages and fed and watered ad libitum for 4–6 days before the experiment.

Chronic spinal preparations. Five rats were spinalized under isoflurane anesthesia. After a laminectomy was performed at the T₁₃ level, the dura and spinal cord were cut and gelfoam was inserted between the cut ends. The skin and muscle incisions were closed in layers with 5–0 chromic gut and 2–0 silk suture, respectively. The bladders of the rats were expressed twice per day for a period of 21–28 days.

Isolated bladder and urethra preparation. Nine additional rats were surgically prepared in the manner described in General preparation and transvesical infusion for transvesical infusions except that the bladder neck was ligated with 5–0 silk suture to create separate bladder and urethral compartments. In six of these animals the urethra was catheterized with PE-50 tubing for the purpose of infusing either saline or acetic acid. Pressure-response curves were generated by attaching the urethral catheter to a three-way valve that allowed connections to both the pressure transducer and a fluid reservoir (2.5 cm diameter) that was positioned at incremental heights above the urethra to generate a constant pressure head. In two preliminary experiments the catheter was tied in place at the urethral meatus to prevent fluid from escaping from the urethra. However, it was discovered that pressure applied to the distal urethra (e.g., by the ligature used to hold the catheter in place) caused inhibition of bladder activity. Because the pressure-response relationship between the urethra and bladder was an objective of this study, the urethral catheter was not tied at the distal urethra in the four experiments. This allowed small volumes of fluid to escape from the urethra, but the volume was insignificant in comparison to the amount of fluid in the reservoir and did not significantly influence the recorded pressure head.

pH measurement. The pH of the acetic acid solution was measured before and after transvesical infusion. Acetic acid released from the bladder (i.e., through micturition reflexes activated by transvesical infusion) was captured immediately following its release from the urethra by a plastic collecting device placed under the ditoris. The pH was measured with litmus paper (Fisher Scientific, Pittsburgh, PA). Data were quantified categorically as below pH 4 or above pH 6. Differences in proportion of samples in each category were evaluated statistically using Fisher's exact (χ²) test.

RESULTS

Transvesical infusion preparation. Anal sphincter EMG recordings were comprised of two components (Fig. 1A). The first component was comprised of short-duration (2 ms) action potentials that were sensitive to the neuromuscular blocking agent succinylcholine (Fig. 1B), indicating that the potentials represented striated anal sphincter activity. It was this striated sphincter activity that was related to rhythmic bladder activity and has been termed the vesicoanal reflex. The second component was comprised of long-duration (50 ms) potentials that were sensitive to cholinergic muscarinic receptor antagonists (e.g., scopolamine methyl bromide, Fig. 1C), indicating the potentials arose from longitudinal smooth muscle cells.

These long-duration, smooth muscle potentials were much fewer in number and were usually of smaller amplitude than the striated muscle potentials. The smaller amplitude and longer duration allowed the vast majority of these smooth muscle potentials to be discriminated by the ratemeter and were not counted during quantification of the striated muscle potentials of interest (Fig. 1C, which shows unusually large-amplitude, scopolamine-sensitive smooth muscle potentials, was from an exceptional experiment selected to
animals exhibited a significant ($P < 0.001$) increase in the striated muscle component of the anal sphincter EMG associated with bladder contractions (i.e., the vesicoanal reflex). The increased activity was asynchronous and was recorded as increases in both firing rate ($432 \pm 114\%$ of control) and duration of firing subsequent to each bladder contraction ($347 \pm 82\%$ of control). In other words, under saline infusion conditions, the vesicoanal reflex activity (when present, see Figs. 5A and 6A) ended simultaneously with the end of the bladder contraction, whereas, during acetic acid infusion, the anal sphincter activity remained elevated for prolonged periods of time after the bladder contraction had ended. In those cases where anal sphincter activity remained elevated for the entire period between bladder contractions (i.e., when bladder pressure was at baseline), an inhibition of anal sphincter activity occurred during the onset of the bladder contraction (compare with Fig. 2A).

In 8 of the 15 animals, an initial robust increase in the vesicoanal activity was recorded immediately on acetic acid reaching the bladder (e.g., Fig. 2A). This initial robust increase gradually diminished over a 10-min period to a maintained, steady-state level (Fig. 2B) that was significantly higher than during saline infusion. In the other seven animals, the onset of the increase was more gradual and required ~20 min to reach a maintained elevated baseline. A return to saline infusion of the bladder reduced anal sphincter activity nearly to original levels (Fig. 2C).

Figure 3A, a high-speed tracing, shows the relationship between a bladder contraction and anal sphincter activity in greater detail. Note some increase in anal sphincter activity occurs during the peak of the initial bladder contraction, which corresponds to the time of urine release (bracketed by arrows). It is during this time of urine release when urethral sphincter activity occurs as “bursts” (15), which are reflected as “high-frequency oscillations” (17) in the bladder pressure recording (i.e., thickening of the bladder pressure tracing at the time bracketed by the arrows). The abrupt increase in anal sphincter activity and bladder pressure coincides with changes in urethral sphincter activity from a bursting to an asynchronous pattern. A previous study indicates that the increase in bladder pressure is due to the asynchronous activity of the urethral sphincter (5). The increase in bladder pressure lasts ~1 min, whereas the increase in anal sphincter activity lasts ~3 min. In summary, under conditions of acetic acid, both the rate and duration of anal sphincter EMG activity associated with a bladder contraction significantly ($P < 0.001$) increased (Fig. 4).

Similar to anal sphincter activity, urethral sphincter activity was also increased by infusion of acetic acid in all 15 animals (Figs. 3B and 4). During saline infusion, urethral sphincter EMG activity was most prominent during the peak of the bladder contraction (i.e., during periods of urine release) and occurred in rhythmic, high-frequency bursts as previously described (7, 15, 18).

Figure 3B, a high-speed tracing, shows the relationship between a bladder contraction and urethral sphincter activity in greater detail. Note some increase in urethral sphincter activity occurs during the peak of the initial bladder contraction, which corresponds to the time of urine release (bracketed by arrows). It is during this time of urine release when urethral sphincter activity occurs as “bursts” (15), which are reflected as “high-frequency oscillations” (17) in the bladder pressure recording (i.e., thickening of the bladder pressure tracing at the time bracketed by the arrows). The abrupt increase in anal sphincter activity and bladder pressure coincides with changes in urethral sphincter activity from a bursting to an asynchronous pattern. A previous study indicates that the increase in bladder pressure is due to the asynchronous activity of the urethral sphincter (5). The increase in bladder pressure lasts ~1 min, whereas the increase in anal sphincter activity lasts ~3 min. In summary, under conditions of acetic acid, both the rate and duration of anal sphincter EMG activity associated with a bladder contraction significantly ($P < 0.001$) increased (Fig. 4).

Figure 3C, a high-speed tracing, shows the relationship between a bladder contraction and urethral sphincter activity in greater detail. Note some increase in urethral sphincter activity occurs during the peak of the initial bladder contraction, which corresponds to the time of urine release (bracketed by arrows). It is during this time of urine release when urethral sphincter activity occurs as “bursts” (15), which are reflected as “high-frequency oscillations” (17) in the bladder pressure recording (i.e., thickening of the bladder pressure tracing at the time bracketed by the arrows). The abrupt increase in anal sphincter activity and bladder pressure coincides with changes in urethral sphincter activity from a bursting to an asynchronous pattern. A previous study indicates that the increase in bladder pressure is due to the asynchronous activity of the urethral sphincter (5). The increase in bladder pressure lasts ~1 min, whereas the increase in anal sphincter activity lasts ~3 min. In summary, under conditions of acetic acid, both the rate and duration of anal sphincter EMG activity associated with a bladder contraction significantly ($P < 0.001$) increased (Fig. 4).

Figure 3D, a high-speed tracing, shows the relationship between a bladder contraction and urethral sphincter activity in greater detail. Note some increase in urethral sphincter activity occurs during the peak of the initial bladder contraction, which corresponds to the time of urine release (bracketed by arrows). It is during this time of urine release when urethral sphincter activity occurs as “bursts” (15), which are reflected as “high-frequency oscillations” (17) in the bladder pressure recording (i.e., thickening of the bladder pressure tracing at the time bracketed by the arrows). The abrupt increase in anal sphincter activity and bladder pressure coincides with changes in urethral sphincter activity from a bursting to an asynchronous pattern. A previous study indicates that the increase in bladder pressure is due to the asynchronous activity of the urethral sphincter (5). The increase in bladder pressure lasts ~1 min, whereas the increase in anal sphincter activity lasts ~3 min. In summary, under conditions of acetic acid, both the rate and duration of anal sphincter EMG activity associated with a bladder contraction significantly ($P < 0.001$) increased (Fig. 4).
17). After the peak of the contraction, the urethral EMG activity became asynchronous and faded as the bladder pressure returned to baseline (Fig. 3B).

Under conditions of acetic acid infusion, there was a facilitation of the bursting urethral EMG activity that occurred during urine release at the initial peak of bladder pressure, but more importantly there was a marked increase in the asynchronous activity that occurred after the initial peak of the bladder contraction, as bladder pressure was falling (Fig. 3B). The effects of acetic acid infusion on urethral sphincter EMG activity are similar to those reported under conditions of "cold stimulation" of the bladder (5). The acetic acid-induced increase in asynchronous urethral sphincter activity was temporally correlated with the increase in large amplitude anal sphincter EMG activity. As previously described for asynchronous urethra EMG activity in chronic spinal cord-transected rats (15) and in rats whose bladders were infused with cold saline (5), this asynchronous activity often resulted in large increases in bladder pressure subsequent to the initial micturition contraction [e.g., compare Figs. 2A and 3A of the current study and Fig. 2C in Cheng et al. (5)]. The increase in bladder pressure presumably resulted from the increase in urethral resistance caused by the asynchronous sphincter activity.

Fig. 2. A: example of acetic acid-induced increase in striated anal sphincter and bladder activity. Note increase in bladder contraction amplitude and appearance of robust anal sphincter activity associated with each contraction, i.e., vesicoanal reflex activity, after switching from 0.9% saline to 0.5% acetic acid. B: example of baseline activity level 10 min after beginning infusion of acetic acid. C: example of marked decrease in vesicoanal reflex activity resulting from a return to saline infusion. Vertical calibration bar = 22 cmH2O in A and B and 9 cmH2O in C for bladder pressure record and 55 µV in A, B, and C for EMG record. Horizontal calibration bar = 1 min.

Fig. 3. A: high-speed records showing relationship between anal sphincter activity (top record) and bladder contraction (bottom record; see text for details). B: comparison of effects of acetic acid infusion (started at arrow below bladder pressure record) on urethral and anal sphincter activity. Note that during saline infusion (to left of arrow) anal activity is sparse. A short time following a switch from saline to acetic acid infusion (arrow), anal sphincter activity robustly increases. In addition, acetic acid increases duration of urethral sphincter activity as well as duration of bladder pressure elevation. Vertical calibration bar for bladder pressure records = 30 cmH2O in A and 50 cmH2O in B and for EMG records = 20 µV in A and 40 µV in B. Horizontal calibration bar = 30 s in A and 1 min in B.
Because of the high level of bladder-related urethral sphincter EMG activity during saline infusion, the acetic-induced increases in urethral sphincter activity were not as robust as acetic acid-induced increases in anal sphincter activity (Figs. 3B and 4), but were statistically significant ($P < 0.05$). The increase in urethral sphincter activity was seen as an increase in both the firing rate ($184 \pm 26\%$ of control) and duration of firing ($190 \pm 32\%$ of control) that accompanied each bladder contraction (Fig. 4). Infusion of acetic acid also decreased bladder capacity to $32 \pm 19\%$ of control measured during the initial 20-min period of infusion.

Rhythmic bladder contractions and vesicoanal reflex activity were not maintained during continuous transvesical infusion of acetic acid. In 18 of 20 animals, bladder contractions and the associated anal sphincter activity gradually decreased beginning $\approx 1$ h after continuous infusion of acetic acid and eventually disappeared. The average time from start of acetic acid infusion to disappearance of bladder contractions was $93 \pm 18$ min. After the bladder and anal sphincter activity had disappeared, the return to saline infusion did not cause a return of bladder activity. Also, administration of the opioid antagonist naloxone (0.1–10 mg/kg iv, $n = 3$) did not cause a return of the activity (data not shown). Chronic infusion of saline into the bladder produces consistent bladder contractions for at least 4 h (17).

Topical application of capsaicin. The application of capsaicin (Fig. 5) directly to the serosal surface of the saline-filled bladder induced a bladder contraction in all seven animals studied. In five of these animals (Fig. 5B) there was also an increase in vesicoanal activity ($304 \pm 136\%$ of saline-infused control activity). Shortly after the application of capsaicin ($\approx 20$ min), bladder activity and subsequently anal sphincter activity disappeared.

In contrast to the effect of capsaicin on a saline-infused bladder, in the acetic acid-infused bladder capsaicin produced no further increase in anal sphincter activity.
ter activity (i.e., above that produced by acetic acid infusion) and, instead, produced a decrease in vesicoanal activity (Fig. 6B). Shortly (~20 min) after the vesicoanal reflex activity disappeared, rhythmic bladder activity also disappeared.

Capsaicin pretreatment. Animals pretreated with capsaicin 4–6 days before the experiment (Figs. 7 and 8) showed significantly less increase in vesicoanal activity during acetic acid infusion (150 ± 30% of saline control activity, n = 6) compared with vehicle-treated animals (367 ± 107% of saline control activity, n = 8) or untreated animals (432 ± 114% of saline control activity, n = 15). Unlike in the untreated or vehicle-pretreated animals, the infusion of acetic acid did not increase the micturition contraction frequency in the animals pretreated with capsaicin. One interesting finding was that rhythmic bladder contractions were obtained from only 6 of the 10 animals pretreated with capsaicin, whereas all vehicle-pretreated animals exhibited contractions. The absence of bladder contractions was seen in both Sprague-Dawley (2 of 6) and Wistar (2 of 4) rats using two different lots of capsaicin.

Chronic spinal preparations. In four of five spinal-transected rats that exhibited rhythmic bladder contractions, infusion of acetic acid in the bladder (Fig. 9) produced a remarkable increase (130 ± 38-fold, i.e., 13,000% of control saline infusion) in vesicoanal reflex activity. External urethral sphincter activity also increased markedly, but still to a lesser extent than anal sphincter activity (22 ± 6-fold, i.e., 2,200%). Bladder

**Fig. 6.** Example of acetic acid-induced vesicoanal reflex (A; arrow indicates start of acetic acid infusion) and subsequent attenuation of this activity after topical application of capsaicin to serosal surface of bladder (B; arrow indicates application of capsaicin). Vertical calibration bar = 34 µV for EMG record and 7 cmH₂O for bladder pressure record. Horizontal calibration bar = 1 min.

**Fig. 7.** Comparison of anal sphincter EMG activity during infusion of saline (A) and acetic acid (B) into bladder of capsaicin-pretreated animal. Vertical calibration bar = 25 µV for EMG record and 10 cmH₂O for bladder pressure record. Horizontal calibration bar = 24 s.
activity during saline infusion was quite variable in the spinal animals, and acetic acid produced an enhancement of the activity (Fig. 9). Because of the inability to record bladder contractions reliably during saline infusion, the increase in bladder activity during acetic acid infusion could not be quantified (i.e., infinite increases are calculated when control bladder activity was zero).

Isolated bladder and urethra preparation. Because the method of transvesical infusion of fluids into the bladder allows the fluid to come in contact with both the bladder and the urethra during a micturition contraction, it is possible that the increased anal sphincter activity and eventual loss of bladder activity are due to acetic acid-induced irritation of the urethra rather than irritation of the bladder. Therefore, the bladder and urethra were separated by a ligature, and the effect of selective exposure of the urethra and bladder to acetic acid was examined on bladder and anal sphincter activity.

Infusion of acetic acid into the isolated bladder produced only a modest increase in anal sphincter activity (156 ± 15% of control) in only two of seven animals. In the other five of seven animals, there was virtually no anal sphincter activity associated with bladder contractions (Fig. 10A). In addition, infusion of acetic acid into the isolated bladder produced no loss of bladder activity for as long as 180 min after infusion of acetic acid into the isolated bladder (i.e., twice as long as the mean time and longer than the longest individual time for loss of bladder contractions during transvesical infusion of acetic acid). After 180 min of isolated bladder exposure to acetic acid without loss of bladder activity, the ligature separating the bladder and urethra was removed and acetic acid was allowed to pass from the bladder into the urethra (i.e., same conditions as original transvesical infusion). Within 68 ± 21 min, bladder activity disappeared in all animals. The weak effects of acetic acid infusion into the isolated bladder compared with the transvesical infusion of acetic acid are not due to less mucosal insult, because histopathological analysis showed similar amounts of mild inflammation in both the isolated bladder and transvesical preparations (data not shown).

In contrast to the weak effects of acetic acid infusion into the isolated bladder, infusion of acetic acid into the isolated urethra produced a robust increase in anal sphincter activity (1,170 ± 229% of control) in three of four animals (Fig. 10B). Furthermore, it was noted that simply inserting a catheter into the urethra and slight movements of the catheter produced robust increases in anal sphincter activity (Fig. 10C). Even infusion of saline into the urethra, in volumes as small as 30 µl, produced robust increases in anal sphincter activity (Fig. 10D). Anal sphincter activation produced by fluid injections into the urethra were reproducible and showed little desensitization.

Capsaicin injection into the urethra also activated the urethroanal reflex (to 847 ± 72% of control, Fig. 10E), and capsaicin pretreatment of the animals 7 days before infusion of acetic acid into the urethra markedly attenuated the evoked anal sphincter activity (to 223 ± 86% of control, Fig. 10F). Both acetic acid infusion and capsaicin injection into the urethra produced an inhibition of bladder activity in all animals (e.g., Figs. 10B and 11A).

Fig. 8. Capsaicin-pretreated animals exhibit less of an increase in anal sphincter activity during acetic acid infusion than do untreated or vehicle-treated animals. Values represent activity recorded during acetic acid infusion expressed as % change from saline infusion controls. *Significant (P < 0.05) compared with untreated group. Error bars = SE; n, no. of rats.

Fig. 9. Example of acetic acid-induced anal sphincter activity in a chronic spinal cord-transected animal. Switching from saline infusion of bladder (A) to acetic acid infusion (B), there is a robust increase in anal sphincter activity and a marked increase in bladder activity. Vertical calibration bar = 55 µV for EMG records and 7 cmH₂O for bladder pressure records. Horizontal calibration bar = 1 min.
On the basis of the effects of urethral infusion, a series of experiments was conducted to determine the pressure-response characteristics of the excitatory urethral reflex activity and the inhibitory urethrovésical reflex activity. As shown in Fig. 11, A and B, anal sphincter activity increased proportionally with increased urethral pressure applied with saline solution up to a pressure of 100 cmH₂O. The increase in anal sphincter activity showed only gradual declines if the pressure elevation was maintained for a greater than 5-min period. Increases in urethral pressure also produced inhibition of rhythmic bladder contractions (Fig. 11A).

When urethral pressure was applied with acetic acid solution instead of saline, the pressure-response curve for anal sphincter activity was much steeper (P < 0.05 for difference between saline and acetic acid at 50 cmH₂O pressure, other differences not significant, n = 3), suggesting that acetic acid sensitized the urethral afferent fibers or that the effects of chemical and mechanical stimulation were additive (Fig. 11B).

Under saline conditions, bladder inhibition occurred at an average intraurethral pressure of 50 cmH₂O, whereas under acetic acid conditions, bladder inhibition occurred at an intraurethral pressure of 30 cmH₂O. Inhibition of the bladder contractions occurred in an all-or-none manner.

pH measurements. Because the response of the urethra to acetic acid exposure was greater when the acetic acid was directly infused into the urethra (i.e., in the isolated bladder and urethra preparations) compared with transvesical infusion, the pH of the acetic acid was compared before and after transvesical perfusion. In all acetic acid solutions tested (n = 10), the pH was less than four before infusion and greater than six after infusion.

Fig. 10. A: acetic acid (0.5 ml of 0.5%) infusion into bladder that was isolated from urethra by a ligature tied at bladder neck. Note only weak vesicoanal activity. B: acetic acid (30 µl of 0.5%) infusion into urethra that was isolated from bladder by a ligature tied at bladder neck. Note robust urethral reflex activity. C: catheter (PE-10) introduction into urethra produces a marked activation of anal sphincter. D: saline infusion (30-µl bolus) into urethra activates anal sphincter. E: capsaicin infusion (30 µl of 1 mg/ml) into urethra activates anal sphincter, and bladder contraction that follows urethral injection activates vesicoanal reflex. Vertical calibration bar = 55 µV for EMG records and 7 cmH₂O for bladder pressure records and horizontal calibration bar = 1 min (for A–E). F: comparison of effects of acetic acid infusion (30 µl of 0.5%) into urethra of untreated, vehicle-pretreated, or capsaicin-pretreated animals. Error bars = SE. * in F indicates P < 0.05.
These results indicate the presence of a nociceptive reflex of acetic acid into the urethra via transvesical infusion. Additionally, direct infusion of acetic acid into the bladder and inconsistently activated anal sphincter activity. (isolated bladder and urethra preparations) only weakly selective instillation of acetic acid into the bladder the presence of a vesicoanal reflex. On the other hand, application of capsaicin directly to the bladder verifies afferent neurons (15).

The vesicoanal and urethroanal synaptic connections have been identified in cats using single-unit and intracellular electrophysiological techniques (10, 11). In humans, it has been reported that rapid distension of the bladder with volumes >50 ml evokes EMG activity of the levator ani muscle (26). Whether bladder irritation would influence this response in humans is not known. One might speculate that one purpose of a vesicoanal reflex initiated by bladder irritation would be to prevent fecal incontinence due to an increase in abdominal pressure during Valsalva maneuvers aimed at expulsion of irritative substances from the lower urinary tract, or it may be a nonspecific reaction to pelvic pain.

In addition to influences on anal sphincter activity, lower urinary tract irritation also influenced urethral sphincter activity. The increase in urethral sphincter activity was not as great as the increase in anal sphincter activity when expressed as a percentage of the control activity. However, this may reflect the very low levels of anal sphincter activity that accompanied bladder contractions during saline infusion compared with the very high levels of activity that are typically seen in urethral activity during a bladder contraction. Both under saline and acetic acid conditions, the urethral sphincter activity that was associated with micturition contractions and urine release was accompanied by multiple high-frequency bursts of urethral sphincter activity. It has been shown that these high-frequency oscillations of the striated muscle of the urethra are dependent on supraspinal structures and are actually necessary for efficient micturition to occur in the rat (7, 15, 17). Whereas the bursting or high-frequency oscillation activity of the urethral sphincter was increased by acetic acid infusion, the more pronounced effects were on the asynchronous urethral sphincter EMG activity. This is similar to the effects produced by cold simulation of the bladder (5). It is likely to be significant that both these models of lower urinary tract irritation produce urethral sphincter activity that is similar to that recorded in chronic spinal cord transected animals (15), in which bladder sphincter dyssynergia prevails.

**DISCUSSION**

The present study has shown that irritation of the lower urinary tract produces activation of somatic anal sphincter motoneurons via a spinal reflex mechanism. These bladder-induced and urethra-induced activations of the somatic anal sphincter motoneurons were termed vesicoanal and urethroanal reflexes, respectively. The vesicoanal and urethroanal reflexes were shown to be initiated by, and dependent on, activation of capsaicin-sensitive fibers, a hallmark of nociceptive afferent neurons (15).

On one hand, anal sphincter activity induced by application of capsaicin directly to the bladder verifies the presence of a vesicoanal reflex. On the other hand, selective instillation of acetic acid into the bladder (isolated bladder and urethra preparations) only weakly and inconsistently activated anal sphincter activity. Additionally, direct infusion of acetic acid into the urethra produced more robust responses than infusion of acetic acid into the urethra via transvesical infusion. These results indicate the presence of a nociceptive vesicoanal reflex, in addition to the vesicoanal reflex. The weaker effects of acetic acid following transvesical infusion may be due to the buffering capacity of the bladder (i.e., acetic acid solutions at pH 4 were infused into the bladder but were released as pH 6 solutions), which may reduce the stimulus properties of the solution. This buffering function of the bladder was unexpected and, together with unexpected findings of significant fluid absorption from rat bladder (27), raises the issue of whether the bladder only serves to store and release urine or if it has additional metabolic functions. In addition to chemical (i.e., capsaicin or acetic acid) sensitivity of the urethroanal reflex, it was also extremely sensitive to modest elevations in pressure or to distension of the urethra with a small fixed volume.

Vesicoanal reflexes induced by acetic acid infusion of the bladder have also been seen in the guinea pig (M. A. Katofiasc and K. B. Thor, unpublished observation) but have not been seen in the cat (Katofiasc and Thor, unpublished observation). However, nonnociceptive vesicoanal and urethroanal synaptic connections have been identified in cats using single-unit and intracellular electrophysiological techniques (10, 11). In humans, it has been reported that rapid distension of the bladder with volumes >50 ml evokes EMG activity of the levator ani muscle (26). Whether bladder irritation would influence this response in humans is not known. One might speculate that one purpose of a vesicoanal reflex initiated by bladder irritation would be to prevent fecal incontinence during increased abdominal pressure during Valsalva maneuvers aimed at expulsion of irritative substances from the lower urinary tract, or it may be a nonspecific reaction to pelvic pain.

In addition to influences on anal sphincter activity, lower urinary tract irritation also influenced urethral sphincter activity. The increase in urethral sphincter activity was not as great as the increase in anal sphincter activity when expressed as a percentage of the control activity. However, this may reflect the very low levels of anal sphincter activity that accompanied bladder contractions during saline infusion compared with the very high levels of activity that are typically seen in urethral activity during a bladder contraction. Both under saline and acetic acid conditions, the urethral sphincter activity that was associated with micturition contractions and urine release was accompanied by multiple high-frequency bursts of urethral sphincter activity. It has been shown that these high-frequency oscillations of the striated muscle of the urethra are dependent on supraspinal structures and are actually necessary for efficient micturition to occur in the rat (7, 15, 17). Whereas the bursting or high-frequency oscillation activity of the urethral sphincter activity was increased by acetic acid infusion, the more pronounced effects were on the asynchronous urethral sphincter EMG activity. This is similar to the effects produced by cold simulation of the bladder (5). It is likely to be significant that both these models of lower urinary tract irritation produce urethral sphincter activity that is similar to that recorded in chronic spinal cord transected animals (15), in which bladder sphincter dyssynergia prevails.

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**Fig. 11.** A: example of increased anal sphincter activity produced by step-wise increases in urethral pressure applied hydraulically with saline. Note increase in anal activity with increase in applied pressure. Also note inhibition of bladder activity at 50 cmH2O pressure. Vertical calibration bar = 55 µV for EMG records and 7 cmH2O for bladder pressure records. Urethral pressure indicated on record. Horizontal calibration bar = 1 min. B: pressure-response characteristics (n = 3) of anal sphincter activation produced by increasing pressure applied with saline or 0.9% acetic acid. Error bars = SE.
In addition to demonstrating nociceptive vesicoanal and vesicourethral sphincter reflexes, the present studies also demonstrated nociceptive urethroanal and urethrourethral sphincter reflexes. Both chemical irritation (by acetic acid or capsaicin infusion) and mechanical irritation (by small-volume distension or modest elevations in pressure) produced increases in anal and urethral striated sphincter activity. The increase in anal activity has not been previously reported, but a previous study has shown that capsaicin infusion into the urethra resulted in activation of striated urethral sphincter activity, which rapidly desensitized (7). In the present study, anal sphincter activation by urethral distension showed no desensitization to repeated injections of small volumes of fluid but did show some accommodation during maintained elevations of constant pressure.

In contrast to the excitatory effects of urethral nociceptive stimuli on anal and urethral sphincter activity, urethral irritation inhibited bladder activity, i.e., an inhibitory urethrovesical reflex. The method of inducing urethral irritation in the present study was relatively mild (i.e., dilute acetic acid infusion, distension with small volumes of saline, catheter movement, etc.), yet it still completely inhibited bladder activity. Similar inhibition of the bladder has been reported following a more severe irritation of the urethra, i.e., capsaicin infusion (8). In the present studies, we showed that the effects of urethral irritation produced by acetic acid infusion or saline distension were mediated by capsaicin-sensitive afferent fibers.

The extreme sensitivity of the urethra to even mild irritation, distension, or elevated pressure suggests that naturally occurring pathophysiological conditions might be capable of activating nociceptive urethral afferent fibers and result in inhibition of bladder activity and activation of anal and urethral striated muscle activity. It may be relevant to note that the clinical conditions of prostatitis and urethritis are oftenaccompanied by increased activation of bladder C fibers, activity seen in patients suffering from certain neurological diseases.

In the present experiments, capsaicin pretreatment was utilized to ascertain the role of small-diameter nociceptive afferent fibers in mediating the various reflexes that accompany lower urinary tract irritation. In light of previous literature that describes only subtle changes in micturition reflexes in capsaicin-pretreated rats (19, 21, 25), it was surprising to find in the present study that only about one-half of the capsaicin-pretreated animals exhibited micturition contractions at all. Furthermore, in those animals that did demonstrate micturition contractions, the bladder capacity was much greater. Differences in strains of rats or lot shipments of capsaicin are unlikely to account for the differences in results between the current studies and previous studies because various strains and multiple lots of capsaicin were examined in the current study.

Importantly, results described in a subsequent paper, from the same laboratory that initially described only subtle changes in the micturition reflexes of capsaicin-pretreated rats, indicated that capsaicin pretreatment, abolished micturition contractions in a large percentage of the animals tested (12, 20). Because one laboratory found only subtle effects, another laboratory found subtle effects at one time point (19, 21, 25) and marked effects at another time point (12, 20), and the current study found only marked effects of capsaicin pretreatment on micturition reflexes, it seems likely that there are unknown variables in the effects of capsaicin on micturition reflexes. Thus the dogma in the literature that capsaicin pretreatment has little effect on micturition contractions in anesthetized normal (i.e., otherwise untreated) animals should be reconsidered.

In summary, the present studies have described various physiological responses that are generated by mild irritation of the lower urinary tract that may mimic naturally occurring pathophysiological conditions. These reflexes may have important clinical implications for the diagnosis and treatment of disorders of the lower urinary tract. Finally, it is proposed that these reflexes may provide a valuable model for the experimental study of physiological and pharmacological regulation of lower urinary tract inflammation.

Perspectives

An important contribution of this paper is likely to be in the use of the vesicoanal reflex as a pharmacological model for examination of drugs that might suppress nociceptive inputs from the lower urinary tract to the spinal cord without affecting normal sensory inputs (28). Such drugs would be useful for reduction of bladder overactivity without inducing urinary retention. The use of bladder activity as an endpoint of a
drug's effects on irritative stimulation of the bladder does not allow one to determine if the drug's actions were specifically reducing urinary tract nociception or bladder contractility. For example, an "anticholinergic" agent would suppress irritation-induced bladder activity, but it could do so without having any effect on nociceptive input. Thus having anal sphincter activity as an independent measure of lower urinary tract irritation allows one to examine a drug's potential for reducing urinary tract nociception without affecting normal bladder function.

Whereas monitoring anal sphincter activity in the rat is a valuable first step in evaluation of a drug's potential for reducing lower urinary tract nociception, the authors emphasize that other species should also be examined. The differences in physiological control of bladder and sphincter between rodents and most other species is large. For example, there have been no descriptions of high-frequency bursting of the urethral sphincter EMG during micturition in humans. Furthermore, the role of C fibers in controlling lower urinary tract function is also different in rodents and other species. Thus the authors question the applicability of results in rodents to higher species but recognize their place as a starting point to reduce usage of more sentient species. Clinical urodynamic studies are needed to evaluate the importance of vesicoanal, urethroanal, and urethrovaginal reflexes in lower urinary tract function and dysfunction in humans.

Address for reprint requests and other correspondence: K. B. Thor, Genupro, Inc., 3900 Paramount Parkway, Morrisville, NC 27560 (E-mail: karl.thor@genupro.ppd.com).

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