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Roles of glutamatergic and serotonergic mechanisms in reflex control of the external urethral sphincter in urethane-anesthetized female rats

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Abstract

This study was conducted to examine reflex mechanisms that mediate urinary bladder and external urethral sphincter (EUS) coordination in urethane-anesthetized female Sprague-Dawley rats. We investigated the properties of EUS reflexes elicited by electrical stimulation of pelvic nerve afferent axons (pelvic-EUS reflex). The changes in the reflexes induced by bladder distension and administration of agonists or antagonists for glutamatergic or serotonergic receptors were examined. The reflexes consisted of an early response (ER, 18- to 22-ms latency) and a late, long-duration (>100-ms latency) response (LR), which consisted of bursts of activity at 20- to 160-ms interburst intervals. In a few experiments, a reflex with an intermediate (40- to 70-ms) latency was also identified. With the bladder empty, the ER, but not the LR, was detected in the majority of experiments. The LR was markedly enhanced when the bladder was distended. The ER remained, but the LR was abolished, after spinal cord transection at T₈–T₉. The ER and LR were significantly decreased 75 and 35%, respectively, by the *N*-methyl-D-aspartate receptor antagonist MK-801 (0.3 mg/kg iv), but only decreased 18 and 14%, respectively, by the α -amino-5-methylisoxazole-4-propionate receptor antagonist LY-215490 (3 mg/kg iv). The serotonin (5-HT_{1A}) receptor agonist 8-hydroxy-2-(di-*n*-propylamino)-tetralin (1 mg/kg iv) enhanced spontaneous EUS activity and the pelvic-EUS reflex. WAY-100635 (0.1–1 mg/kg iv), a 5-HT_{1A} antagonist, reversed the effect of 8-hydroxy-2-(di-*n*-propylamino)-tetralin and suppressed EUS activity and the pelvic-EUS reflex. These results indicate that glutamatergic and serotonergic mechanisms are important in the reflex pathways underlying bladder-sphincter coordination in rats.

Keywords

pelvic nerve; bladder distension; bursting activity; spinal cord injury

The lower urinary tract (LUT) has two main functions: storage and periodic elimination of urine. These functions are regulated by a complex neural control system, located in the brain and spinal cord, that coordinates the activity of the two components of the LUT: the

reservoir (urinary bladder) and the outlet (bladder neck, urethra, and urethral sphincter). Normally, these two components exhibit reciprocal activity (26). During urine storage, the reservoir is quiescent, and activity in the outlet gradually increases during bladder filling to maintain continence. During voiding, the activity is reversed.

The LUT is regulated by three sets of peripheral nerves: sacral parasympathetic (pelvic) nerves and thoracolumbar sympathetic (hypogastric) nerves, which innervate bladder and urethral smooth muscles, and sacral somatic (pudendal) nerves, which innervate striated muscles of the external urethral sphincter (EUS) (9, 26, 28). Afferent axons from the bladder that initiate EUS activity during urine storage and voiding travel in the pelvic nerves. Afferent axons from the urethra passing through the pelvic and pudendal nerves can modulate reflexes evoked by bladder afferent nerves (10).

Previous studies in the cat and rat have demonstrated EUS reflex activity or firing of motor axons in the pudendal nerve in response to bladder distension (6, 13, 15, 27, 31) or electrical stimulation of afferent axons in the pelvic nerve (20, 21, 24, 25). Bladder distension at volumes below the threshold for evoking micturition elicits tonic activity of the EUS, whereas bladder distension at volumes sufficient to initiate micturition elicits an inhibition of EUS activity in the cat (3, 13) but prolonged bursting activity of the EUS at frequencies of 6–8 Hz in the rat (4, 18, 22). It has been proposed that bursting reflects rhythmic contractions and relaxations of the EUS, which produces an intermittent flow of urine. A detailed analysis of the EUS bursting pattern has revealed that it consists of silent periods averaging 104 ms and active periods averaging 67 ms (4). EUS bursting, as well as tonic EUS activity, occurs in rats with an intact spinal cord, as well as in rats with chronic T8 spinal cord transection and, therefore, must be mediated by spinal reflex pathways (4). However, it is not known whether the tonic activity and bursting patterns of EUS activity occur via different reflex mechanisms or simply reflect activity generated in the same reflex pathway by different levels of afferent input from the bladder. In the present experiments, we have studied this question by recording reflex activity elicited in the EUS by single-shock electrical stimulation of afferent axons in the pelvic nerve. The influence of bladder distension on the reflexes was also analyzed.

Previous studies revealed that bladder and EUS reflexes are sensitive to *N*-methyl-D-aspartate (NMDA) and α -amino-5-methylisoxazole-4-propionate (AMPA) glutamatergic receptor antagonists (37–41), as well as serotonin (5-HT) receptor agonists and antagonists (16, 30, 32–34), indicating that glutamate and 5-HT are transmitters in the reflex pathways controlling the LUT. The present experiments extended these observations by evaluating the effect of glutamatergic and serotonergic drugs on EUS reflex activity elicited by electrical stimulation of pelvic nerve afferent axons.

MATERIALS AND METHODS

Animal preparation

Female Sprague-Dawley rats ($n = 40$, 250–330 g) were anesthetized with urethane (1.2 mg/kg sc). Supplemental doses of urethane (0.4 mg/kg sc) were given as needed throughout the experiments. The experimental protocols were approved by the University of Pittsburgh Institutional Animal Care and Use Committee.

A polyethylene (PE)-50 tube filled with physiological saline was inserted into the jugular vein for intravenous administration of drugs. After exposure of the urinary bladder via a midline abdominal incision, a PE-50 tube filled with saline was inserted through an incision in the apex of the bladder and secured with cotton thread. Intravesicularly injected saline (0.2–1.0 ml) was used to distend the bladder and pass fluid through the urethra. Because

leakage of fluid from the bladder around the catheter and into the urethra could trigger additional reflexes, in some animals, another bladder catheter with a small balloon attached to the tip was inserted. Air was injected (0.2–0.5 ml) into the balloon to distend the bladder and avoid triggering additional urethral-bladder or urethral-EUS reflexes. The pubic symphysis was removed to expose the urethra and EUS. To record the EUS electromyogram (EMG), two fine, insulated silver wire electrodes (0.05 mm diameter) with exposed tips were inserted into the muscle on both sides of the midurethra, 5–8 mm from the bladder neck. The left pelvic nerve was isolated. By retraction of skin flaps, a pool was formed around the pelvic nerve, bladder, and urethra and filled with mineral oil (37°C) to prevent drying. For electrical stimulation and recording of reflexes, bipolar silver wire electrodes were positioned on the pelvic nerve 3–5 mm central to the major pelvic ganglion. In three animals, contributions of supraspinal pathways to the reflexes and drug effects were evaluated by transection of the spinal cord at T8–T9 1 h after control recordings of the pelvic nerve afferent-evoked reflex (pelvic-EUS reflex). The experimental setup for modulating bladder pressure, as well as for measuring the EUS EMG, is depicted in Fig. 1.

Electrophysiological and pharmacological experiments

The pelvic-EUS reflex was studied before and after bladder distension. Bladder pressure was manipulated by injection of saline (0.2–1.0 ml) or air (0.2–0.5 ml) into the bladder. The pelvic-EUS reflex was elicited by single shocks (Grass S88 stimulator) to the pelvic nerve and recorded from the EUS on both sides. The electrical stimulation consisted of biphasic charge-balanced pulses at frequencies between 0.5 and 3 Hz with a 0.05-ms pulse width. Submaximal stimulus intensities of 4–8 V in different experiments were set in each experiment at 6–10 times threshold for elicitation of a detectable reflex. The evoked pelvic-EUS reflex activity was amplified 20,000-fold with a preamplifier (model P511AC, Grass Instruments) and filtered (high-frequency cutoff at 3,000 Hz, low-frequency cutoff at 3 Hz in combination with a 60-Hz notch filter). The reflex signal was also sampled at 2,500 Hz and then averaged (5–10 reflexes) on a personal computer (PC). The single-sweep and averaged reflex responses were recorded on a PC.

After the electrophysiological studies, pharmacological experiments were conducted according to two protocols for observation of the effects of glutamatergic and serotonergic drugs on EUS EMG activity and EUS reflexes evoked by electrical stimulation. MK-801 (dizocilpine, Sigma), LY-215490 {9(3*SR*,4*aRS*,6*RS*,8*aRS*)-6-[2-(1*H*tetrazol-5-yl)-ethyl]-decahydroisoquinoline-3-carboxylic acid; Lilly Research Laboratories}, WAY-100635 {*N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride; Sigma}, and 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT; Sigma) were dissolved in normal saline for intravenous administration. Drug doses were calculated for the base of each compound. In some experiments, the reflexes were recorded for 30 min before and after single doses of MK-801 (0.3 mg/kg iv) or LY-215490 (3.0 mg/kg iv). Doses were based on results from previous experiments (36, 38, 39). In other experiments, the drugs were administered sequentially: MK-801 1 h before LY-215490 and 8-OH-DPAT 30 min before WAY-100635. In some experiments, the reflexes were recorded for 30 min before and after single doses of 8-OH-DPAT (1.0 mg/kg iv) or WAY-100635 (0.1–1.0 mg/kg iv). Doses were based on results from previous experiments (16, 19, 34).

Data analysis

Spontaneous EUS EMG activity and the late response of the pelvic-EUS reflex were rectified and measured by integration of the area under the curve (in $\mu\text{V}/\text{ms}$). The areas represent 200 ms of spontaneous EUS EMG activity and the first 200 ms of the late response (LR). The areas of the early response (ER) of single pelvic-EUS reflexes were rectified and then measured by integration of the area under the curve (in $\mu\text{V}/\text{ms}$) over the duration of the

reflex responses with a PC (2, 29). Reflex area was more consistent over long periods of time and, therefore, more suitable than reflex amplitude for representing the intensity of reflex activity. The quantitative data represent the reflex area and amplitude of the ER and LR. Twenty single-sweep recordings were analyzed to obtain each data point. Values are means $\pm$ SE. The reflex areas obtained before and after bladder distension, as well as drug administration, were evaluated statistically by using the paired Student's *t*-test. The independent *t*-test was used to compare results in two groups of animals. $P < 0.05$ was considered statistically significant.

RESULTS

Pelvic-EUS reflex

In rats with an intact spinal cord when the bladder was empty, EUS activity evoked by electrical stimulation of the pelvic nerve consisted of a large-amplitude, short-latency (21.6 ± 0.2 ms), short-duration (16.1 ± 1.9 ms) ER, which was detected in every experiment ($n = 40$), and a small-amplitude, long-latency (100- to 120-ms), long-duration (>100 -ms) LR, which was detected in only 30% of the experiments (12 of 40 rats). In a few experiments ($n = 6$), a small intermediate (40- to 70-ms latency) response (IR) was also detected (Fig. 2). The IR was difficult to distinguish from the end of the ER and was often more obvious after administration of drugs that suppressed the LR and spontaneous EUS activity (Fig. 2).

When the bladder was distended with air (0.2–0.5 ml) or saline (0.2–1.0 ml), which elicited small increases in basal EUS activity (Fig. 2, *A* and *B*, *top traces*), the LR was markedly increased in amplitude (Fig. 2*B*), with a much smaller increase or no change (Fig. 2*B*) in the amplitude or the area of the ER. We evaluated two methods of bladder distension, because distension with saline would cause fluid to leak through the urethra and, therefore, might stimulate urethral reflexes, whereas air distension would activate only bladder receptors and, therefore, might elicit different responses. However, the two methods produced similar results, indicating that activation of urethral afferents is not an important factor.

In the presence of bladder distension, the LR was detected in 98% of experiments and usually consisted of a series of burst discharges (10- to 50-ms burst duration and 20- to 160-ms interburst intervals) that could persist for as long as 1.5–3 s (see Fig. 4*A*). The earlier interburst intervals were usually shorter than the later intervals. The interburst interval data for each animal represent the average of the first five intervals for twenty individual evoked responses. The latency for the initial burst was similar to the latency (100–140 ms) of the LR when the bladder was empty, although in some experiments the latency was shorter during bladder distension (cf. Fig. 2). Individual recordings often revealed that the bursts consisted of large-amplitude transient potentials superimposed on low-amplitude longer-duration bursting activity. The large-amplitude potentials did not occur with every burst (Fig. 3*B*). When multiple individual responses were averaged ($n = 8$ –10 records), the large potentials usually were not detected, and only the low-amplitude burst discharges were obvious (cf. Fig. 3, *A* and *D*). In some experiments (10 of 40 rats), the bursting was not very obvious in the averaged records but was evident in individual records (Fig. 3, *A* and *B*). The reflex area and amplitude of the ER and the initial potentials of the LR from the averaged recordings were reasonably consistent (Fig. 3, *C* and *D*) and were used to analyze the effects of bladder distension and drugs. The effect of bladder distension on the LR was reversed by emptying the bladder. The response to bladder distension could be repeated many times in the same animal.

The stimulus threshold for evoking the ER (0.6–0.8 V, 0.05-ms pulse duration) was lower than the threshold for the LR (1.2–1.6 V). The reflexes were not altered by varying the stimulation frequency between 0.5 and 3 Hz ($n = 40$). The amplitudes of the ER and LR

were decreased (16 ± 3.5 and $12.7 \pm 1.2\%$, respectively) when the pelvic nerve was crushed peripheral to the site of stimulation (Fig. 4B) and completely eliminated when the pelvic nerve was crushed central to the site of stimulation (Fig. 4C). At 1 h after spinal cord transection at T8, the area of the ER was significantly suppressed by $40.1 \pm 2.7\%$ ($n = 3$), whereas the LR was completely abolished. The LR did not recover during the remainder of the experiments (up to 4 h).

Spontaneous EUS EMG activity induced by bladder distension was also altered by spinal cord transection. As reported by other investigators (4, 18), before spinal cord transection, bladder distension with small volumes (0.2 ml) induced tonic EUS EMG activity, whereas large volumes (0.4–0.6 ml) induced EUS EMG bursting (5–7 Hz), which coincided with a large-amplitude bladder contraction indicative of a micturition reflex. After spinal cord transection, the bursting was eliminated, but tonic EUS EMG activity still occurred and increased during bladder distension.

Role of glutamatergic mechanisms in reflex pathways

The effects of intravenous administration of glutamatergic receptor antagonists on the pelvic-EUS reflexes were examined when the bladder was distended or empty. MK-801, an NMDA receptor antagonist, in a dose (0.3 mg/kg iv) shown previously (37) to suppress the micturition reflex pathway, markedly suppressed the reflex area (76%, $n = 11$) and amplitude (47%, $n = 11$) of the ER and partially suppressed the LR by 35% (Figs. 5B and 6A). The LR persisted after MK-801 in the few experiments where it was detected. The effects of MK-801 were evident within 10 min and persisted for ≥ 1 h. LY-215490, an AMPA receptor antagonist, administered 30 min after MK-801 in a dose that blocks the micturition reflex (3 mg/kg iv) (40) produced only a minor additional effect on the ER and LR (14 and 18% suppression, respectively, $n = 11$; Figs. 5C and 6A). The effect of the drug was evident 20–30 min after administration. LY-215490 (3 mg/kg iv) alone slightly depressed the ER and LR (18 and 14%, respectively, $n = 7$). The combination of MK-801 (0.3 mg/kg iv) and LY-215490 (3 mg/kg iv) also suppressed the LR by $46.7 \pm 1.4\%$ and spontaneous EUS activity by $39.1 \pm 3.2\%$ ($n = 11$). The effects of the drugs persisted for ≥ 1 h after intravenous administration.

Effects of 8-OH-DPAT on spontaneous and evoked EUS EMG activity

When the bladder was empty, 8-OH-DPAT, a 5-HT_{1A} receptor agonist, administered in a dose (1 mg/kg iv) that is known to enhance reflex bladder activity (11, 19), produced an initial period of EUS EMG bursting activity that occurred 1–2 min after drug injection and persisted for 3–4 min. This was followed by ≥ 40 min of enhanced tonic activity. The tonic EUS activity was significantly enhanced 20–50%; average area under the curve increased from 0.31 ± 0.07 $\mu\text{V/s}$ before 8-OH-DPAT to 0.41 ± 0.01 $\mu\text{V/s}$ after 8-OH-DPAT ($n = 11$). The area of the ER was significantly increased by $16.2 \pm 1.7\%$ ($n = 11$). In 30% of animals in which a small LR was present when the bladder was empty, 8-OH-DPAT produced only a small increase ($6.1 \pm 3.7\%$, $P > 0.05$) in the area of the LR. In the animals in which the LR was absent, 8-OH-DPAT did not unmask the LR.

When the bladder was distended, 8-OH-DPAT significantly increased the area (22%, $n = 11$; Figs. 6B and 7A) and duration of the ER (from 17.8 ± 4.3 to 21.2 ± 6.6 ms, $n = 11$, $P < 0.05$) but did not significantly change the reflex latency (22.7 ± 0.2 vs. 21.9 ± 0.6 ms, $P > 0.05$, $n = 11$). 8-OH-DPAT shortened the LR interburst intervals from 81.9 ± 7.8 ms (range 20–160 ms) in control recordings to 50.3 ± 4.3 ms (range 20–80 ms) and increased the amplitude ($30.2 \pm 5.6\%$, $n = 11$, $P < 0.05$) and area of the LR (85%, $n = 11$; Fig. 6B). After administration of 8-OH-DPAT, the single LR bursts in individual recordings were easily identified (Fig. 7B1); however, in averaged recordings, single bursts of the LR were often

not clear because of shorter interburst intervals and larger amplitude of spontaneous activity (Fig. 7B2). After 8-OH-DPAT, when the bladder was distended, 20% of the recordings also showed a period of inhibition of spontaneous activity after the ER that occurred at a latency of 75–100 ms and a duration of 100 ms.

After spinal cord transection ($n = 3$), when the bladder was empty (Fig. 8A), 8-OH-DPAT elicited a small increase in the ER (14%) and tonic EUS EMG activity (7%) and unmasked an IR in two experiments (Fig. 8A2). When the bladder was distended (Fig. 8B), 8-OH-DPAT also elicited a small increase in the ER (11%) and tonic EUS EMG activity (18%) and unmasked a small LR (Fig. 8B2).

Effects of WAY-100635 on spontaneous and evoked EUS EMG activity

In 8-OH-DPAT-treated, spinal cord-intact rats, WAY-100635, a 5-HT_{1A} antagonist, in a dose (1 mg/kg iv) shown previously (16) to suppress bladder reflexes, markedly suppressed spontaneous EUS EMG activity 60–80% (from $0.41 \pm 0.01 \mu\text{V/s}$ before to $0.19 \pm 0.06 \mu\text{V/s}$ after WAY-100635, $n = 11$) and significantly reduced the ER (59%) and the LR (28%; Figs. 6B and 8, A3 and B3). The effects occurred rapidly, within 30–50 s, and were obtained when the bladder was empty (Fig. 8A3) or distended (Fig. 8B3). WAY-100635 also reversed the effects of 8-OH-DPAT in spinal cord-transected rats.

WAY-100635 administered alone in a small dose (0.1–0.5 mg/kg iv) to spinal cord-intact rats in which the bladder was distended for 5 min by inflation of a balloon catheter suppressed the amplitude and reflex area of the ER by $12.9 \pm 0.5\%$ and the LR by $43.29 \pm 2.6\%$ ($n = 8$). The latency (20.8 ± 0.2 ms, $n = 8$) of the ER was not significantly changed; however, the ER duration was shorter (13.7 ± 1.5 vs. 16.1 ± 1.9 ms, $n = 8$) after WAY-100635. A larger dose (1.0 mg/kg iv) of WAY-100635 suppressed the ER by 63% and the LR by 33% ($n = 8$; Fig. 6B).

The effects of WAY-100635 (0.1–1.0 mg/kg iv) alone on EUS EMG activity induced by transient bladder distension with a balloon catheter or infusion of saline into the bladder were also examined in spinal cord-intact rats. Brief (50-s) distension of a balloon with 0.5 ml of air induced a bimodal EUS discharge consisting of a transient period (i.e., a few seconds) of bursting activity followed by 5–8 s of tonic activity and then a prolonged period of bursting activity that was maintained until the bladder was emptied (Fig. 9). After the bladder was emptied, tonic activity continued for 75–90 s (Fig. 9A). Subsequent infusion of saline (1 ml) into the bladder induced a transient period of bursting followed by tonic activity. The bursting and tonic activity are shown on an expanded time scale in Fig. 9, B and C, respectively. WAY-100635 dose dependently (0.1 and 0.4 mg/kg iv) suppressed EUS EMG activity after a delay of 20–50 s ($n = 2$). The small dose (0.1 mg/kg iv) of WAY-100635 suppressed the amplitude (21%) and duration (38%) of the initial and secondary bursting activity induced by balloon distension and decreased the duration (29%), but not the amplitude, of the tonic phase. The larger dose (0.4 mg/kg iv) of WAY-100635 markedly decreased the duration of bursting (84%) and tonic (61%) EUS EMG activity induced by saline or balloon distension (Fig. 9A).

DISCUSSION

The present study examined several properties of the pelvic-EUS reflex, including organization of the peripheral and central pathways, sensitivity to bladder distension, and responses to pharmacological agents. The experiments revealed that electrical stimulation of afferent axons in the pelvic nerve usually elicits two reflexes in the EUS: one at short latency (average 21.6 ms, ER) and another at long latency (average 110 ms, LR). The two

reflexes are mediated by different pathways, utilize different transmitters, and appear to have different roles in the regulation of micturition.

The ER could be elicited in the absence of the LR by use of low intensities of electrical stimulation (0.6–0.8 V, 0.05-ms duration) with the bladder empty. The LR occurred at slightly higher stimulus intensities (1.2–1.6 V) when the bladder was distended. On the basis of previous electrophysiological studies (7, 23) in our laboratory that established the electrical thresholds for activation of A δ - and C-fiber axons in the pelvic nerve of the rat, it is reasonable to conclude that A δ -fiber afferent axons trigger the early and late pelvic-EUS reflexes.

The central delays for the ER and LR can be estimated by subtraction of peripheral afferent and efferent conduction times from the shortest latencies of the reflexes (average 21.6 ms for ER and 100 ms for LR). With an average conduction distance of 70 mm between the site of stimulation on the pelvic nerve and L6, the shortest afferent conduction time for A δ -fiber afferents with maximal conduction velocities of 10 m/s would be ~7 ms (23). With an efferent conduction time of 8 ms (2, 23), the calculated shortest central delays for the ER and LR would be 6.6 and 85 ms, respectively. It is unlikely that C-fiber afferents can contribute to the ER, because the estimated peripheral afferent conduction time of 70 ms (with the assumption of a 1 m/s conduction velocity and a 70-mm conduction distance) would be longer than the reflex latency. However, the long central delay of the LR would allow it to be modulated by C-fiber afferent input.

The durations of the two major reflexes were also markedly different. The ER had a short (20-ms) duration, whereas the LR consisted of a series of bursts lasting up to 3 s. Individual bursts lasted for 50 ms, and the interval between bursts averaged 81 ms. It is noteworthy that this bursting was similar to the 6- to 8-Hz bursting frequency of the EUS, which occurs during reflex voiding induced by bladder distension. Thus it seems likely that electrical stimulation of bladder afferent axons in the pelvic nerve produces a transient activation of the same central mechanism that controls bursting of the EUS during micturition. Facilitation of the LR by bladder distension is consistent with the idea that mechanosensitive bladder afferents and the afferents activated by electrical stimulation of the pelvic nerves are part of the same population.

The central pathways involved in the ER and LR are also different. Although both reflexes were present in spinal cord-intact rats, the LR, but not the ER, was eliminated after acute spinal cord transection at T8. Thus the ER resembles the tonic EUS activity induced during bladder filling before the onset of micturition. The tonic EUS activity induced by bladder distension persists after acute spinal cord transection and, therefore, must be mediated by spinal reflex pathways (14, 15, 17). This tonic EUS activity induced by bladder distension has been termed the “guarding reflex” and is thought to contribute to urinary continence.

The LR, which may be related to the EUS bursting activity that occurs during voiding, has properties similar to those of the spinobulbospinal micturition reflex elicited on bladder postganglionic nerves by electrical stimulation of afferent axons in the pelvic nerves (23). The two reflexes have long latencies, and both exhibit bursting activity (5, 23). The estimated central delay (60 ms) for the parasympathetic micturition reflex in spinal cord-intact rats (22) is similar to the estimated central delay (85 ms) of the EUS LR. This raises the possibility that the sphincter and parasympathetic autonomic components of micturition are mediated in part by the same central pathways.

The EUS bursting activity during micturition, similar to the EUS LR, is blocked after acute spinal cord transection, indicating that both are linked to the supraspinal mechanisms involved in normal micturition. However, EUS bursting activity reemerges in chronic spinal

cord-transected rats, along with recovery of the micturition reflex (8). Small-amplitude LRs were detected in some spinal cord-transected animals. It will be important to determine in future experiments whether the typical EUS LR also recovers in chronic spinal cord-transected rats. It is noteworthy that long-latency firing elicited on bladder parasympathetic postganglionic nerves during a micturition reflex is also eliminated by acute spinal cord transection (23). Although micturition reflexes return in chronic spinal cord-transected rats, the long-latency firing does not recover. Instead, short-latency bladder-to-bladder reflexes appear in the chronic spinal cord-transected rats. These data indicate that parasympathetic reflexes to the bladder in normal rats are mediated by a spinobulbospinal pathway passing through the pontine micturition center, whereas bladder reflexes in chronic spinal cord-transected rats are mediated by a short-latency spinal pathway (8, 14, 15). It is not possible to determine whether the EUS LR is also mediated by a similar spinobulbospinal pathway or merely facilitated by this pathway during bladder distension.

Previous studies (35–38, 41) revealed that glutamatergic transmission mediated by NMDA and AMPA receptors in the spinal cord and brain is essential for neural control of the LUT. EUS bursting activity during micturition in anesthetized rats can be completely suppressed by blockade of either type of receptor. Thus the present results demonstrating that EUS reflexes are only partially blocked by glutamatergic receptor antagonists and that NMDA glutamatergic mechanisms are more important than AMPA mechanisms in the EUS reflexes were unexpected. A possible explanation for these differences is that reflexes elicited by synchronous afferent volleys in response to electrical stimulation might be more resistant to antagonists than responses induced by slow distension of the bladder during cystometrograms. Electrical stimulation could also change the relative sensitivity of the reflexes to the two types of antagonists. Our studies also showed that the EUS LR was more resistant than the ER to blockade with the NMDA antagonist. This difference might be related to participation of other neurotransmitters in the LR. The greater sensitivity of the ER than the LR to the NMDA antagonist further supports the view that these two reflexes are mediated by different central pathways and that storage reflexes are more susceptible to blockade than voiding reflexes.

EUS bursting activity, which occurs during micturition in the rat, also occurs in the dog, but not in other species, such as the cat and human, in which EUS activity during voiding is inhibited and the urethra completely relaxes. It has been proposed that EUS bursting activity in the rat reflects rhythmic contractions and relaxations of the EUS, which promote the flow of urine. Thus the enhancement of EUS bursting activity by 8-OH-DPAT is consistent with its facilitatory effect on reflex bladder activity and indicates that the drug enhances the visceral and somatic components of voiding in the rat. 8-OH-DPAT also induced EUS bursting activity and elicited a small increase in the EUS LR when the bladder was empty. Thus the drug mimicked the facilitatory effect of bladder distension.

A previous study (19) indicated that 8-OH-DPAT can act at different sites in the central nervous system (i.e., brain and spinal cord) to enhance bladder activity. The 8-OH-DPAT enhancement of EUS bursting activity and the LR was very prominent in spinal cord-intact rats but weak or absent in spinal cord-transected rats, suggesting an action on supraspinal sites. However, other studies (12) have shown that 8-OH-DPAT induces EUS EMG bursting activity and facilitates voiding in chronic spinal cord-injured rats, indicating that the drug has an important facilitatory action on EUS pathways in the spinal cord.

WAY-100635, a 5-HT_{1A} receptor antagonist, blocked the facilitatory effect of 8-OH-DPAT on EUS LR and EUS bursting activity. WAY-100635 administered alone during bladder distension also suppressed the EUS LR, raising the possibility that the EUS LR pathways are tonically facilitated by endogenous 5-HT acting on 5-HT_{1A} receptors. However,

WAY-100635 might also act by influencing 5-HT inhibitory mechanisms (12). It has been speculated that blockade of 5-HT_{1A} autoreceptors on raphe neurons in the brain by WAY-100635 enhances raphe neuron firing, leading to increased release of 5-HT in the spinal cord and inhibition of spinal bladder reflex pathways. The site of action of WAY-100635 will be determined in future experiments by examination of its effects on EUS EMG activity in chronic spinal cord-transected rats in which the bulbospinal serotonergic pathways have been eliminated.

8-OH-DPAT and WAY-100635 also exerted weaker facilitatory and inhibitory effects, respectively, on the EUS ER and tonic EUS activity. This finding is consistent with previous reports that, in some species, 5-HT enhances sphincter activity during urine storage (11, 33). The possibility that 5-HT plays a role in urine storage reflexes prompted an analysis of the effects of duloxetine, a 5-HT-norepinephrine reuptake inhibitor, on LUT function (34). This drug is used clinically to enhance urethral sphincter tone in patients with stress urinary incontinence (34). The 8-OH-DPAT-induced increase in tonic EUS activity when the bladder is empty and facilitation of the EUS ER further support the proposed link between the EUS ER and the bladder-EUS guarding reflex, which promotes continence during bladder filling. Thus it is likely that synapses utilizing 5-HT_{1A} receptors are involved in promoting storage, as well as voiding reflexes.

In conclusion, our experiments have revealed that the ER and LR components of the pelvic-EUS reflexes are mediated by different reflex pathways and that the ER and LR may occur by reflex mechanisms that are responsible, respectively, for the tonic and bursting EUS activity induced by bladder distension. Pharmacological experiments revealed that 5-HT_{1A} and NMDA glutamatergic receptor mechanisms in the central nervous system have an excitatory role in tonic/ER and bursting/LR responses, indicating that 5-HT and glutamic acid are involved in the control of storage, as well as voiding, functions of the LUT.

Acknowledgments

Preliminary results have been presented in abstract form (1).

GRANTS

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Fig. 1.
Experimental setup. EUS, external urethral sphincter; stim and rec, stimulation and recording electrodes.

Fig. 2.

Pelvic EUS reflex was enhanced by bladder distension in spinal cord-intact rats. *A*: EUS electromyogram (EMG) activity without electrical stimulation (*top trace*) and pelvic-EUS reflex elicited by a single shock (7.5 V, 1.0 Hz, 0.05-ms pulse width) to the pelvic nerve (●, *bottom trace*) when the bladder was empty. Reflex responses consisted of a large early response (ER) and small intermediate (IR) and late responses (LR). *B*: EUS EMG activity without electrical stimulation when the bladder was distended by 0.2 ml of saline (*top trace*) and the LR of the pelvic-EUS reflex was enhanced by bladder distension (*bottom trace*). Responses represent average of 10 individual reflexes recorded on a computer.

Fig. 3.

Representative examples of individual and averaged pelvic-EUS reflexes when the bladder was distended by 0.5 ml of air. Individual and averaged reflexes were recorded in the same spinal cord-intact animal. *A*: control spontaneous activity without electrical stimulation (*top trace*) and a single-sweep recording showing the pelvic-EUS reflex, which consisted of an ER and a LR composed of a series of burst discharges at 100- to 150-ms intervals (*bottom trace*). Bursts consisted of low-amplitude long-duration firing and one or two high-amplitude transient potentials. *B*: single-sweep recording showing variability of LR and consistency of ER. *C*: average of 5 individual recordings showing high-amplitude potentials of LR. *D*: average of 10 individual recordings showing that high-amplitude potentials of LR

did not average well, presumably because of variation in latency between individual recordings. However, low-amplitude and long-duration burst activity is still obvious. ●, Electrical stimulation (8 V, 1.0 Hz, 0.05-ms pulse width).

Fig. 4.

Effect of crushing the pelvic nerve peripherally and centrally to the site of stimulation on the pelvic-EUS reflex when the bladder was distended. Each trace represents an individual recording. Individual reflexes were recorded in the same spinal cord-intact animal. *A*: EUS spontaneous activity without electrical stimulation (*top trace*) and pelvic-EUS reflex elicited by electrical stimulation (*bottom trace*). *B*: some high-amplitude potentials of LR were eliminated by crushing the pelvic nerve peripherally to the site of stimulation. *C*: pelvic-EUS reflex was completely eliminated when the pelvic nerve was crushed centrally to the site of stimulation, but spontaneous EUS activity remained, because the contralateral pelvic nerve remained intact. •, Electrical stimulation (6 V, 0.5 Hz, 0.05-ms pulse width).

Fig. 5.

Effects of MK-801 and LY-215490 on the pelvic-EUS reflex. Individual reflexes were recorded in the same spinal cord-intact animal. *A*: pelvic-EUS reflex elicited by a single shock when the bladder was distended. *B*: reduction in amplitude in the pelvic-EUS reflex after administration of MK-801 (0.3 mg/kg iv). *C*: after MK-801, LY-215490 (3 mg/kg iv) suppressed the ER. Responses represent individual reflexes recorded on a computer. ●, Electrical stimulation (5 V, 0.5 Hz, 0.05-ms pulse duration).

Fig. 6.

Effects of drugs on reflex area of the ER and LR in the pelvic-EUS reflex in spinal cord-intact rats. Area of ER was measured when the bladder was empty; area of the LR was measured when the bladder was distended by 0.2 ml of saline. **A:** ER and LR were significantly decreased by 75 and 35%, respectively ($n = 11$, compared with control recordings without drugs) after MK-801 (0.3 mg/kg iv). ER and LR were decreased by 18 and 14%, respectively ($n = 7$, $P > 0.05$ vs. control recordings without drugs) after LY-215490 (3.0 mg/kg iv). When MK-801 was administered first, followed 30 min later by LY-215490, ER and LR were reduced by 87 and 53%, respectively ($n = 11$). **B:** 8-hydroxy-2-(di-*n*-propylamino)-tetralin (8-OH-DPAT, 1.0 mg/kg iv) significantly increased ER and LR by 22 and 85%, respectively ($n = 11$, $P < 0.05$ vs. control recordings without drugs). WAY-100635 (1.0 mg/kg iv) significantly decreased ER and LR by 63 and 33%, respectively ($n = 8$, compared with control recordings without drugs). After 8-OHDPAT,

WAY-100635 significantly suppressed ER and LR by 59 and 28%, respectively ($n = 11$, compared with recordings after 8-OH-DPAT).

Fig. 7.

Effects of 8-OH-DPAT on pelvic-EUS reflex when the bladder was distended (0.5 ml of air). In *A1* and *B1*, *top traces* were obtained without electrical stimulation, and *bottom traces* were obtained during stimulation. Reflexes were evoked by a single shock to the pelvic nerve. Individual and averaged reflexes were recorded in the same spinal cord-intact animal. *A1*: spontaneous EUS activity without electrical stimulation before administration of 8-OH-DPAT (*top trace*) and 2 single sweeps of pelvic-EUS reflex consisting of an ER and some high-amplitude LR bursts (*bottom trace*). *A2*: average of 10 individual recordings showing an ER and a few low-amplitude bursts of LR. *B1*: increase in EUS spontaneous activity without electrical stimulation after administration of 8-OH-DPAT (*top trace*) and unchanged ER and enhanced LR compared with recordings in *A* (*bottom trace*). *B2*: average of 10 individual recordings showing an ER and variable high-amplitude potentials of LR. Because latencies of LR were shifted and variable after 8-OH-DPAT, LR in an average of 10 individual recordings was difficult to identify. ●, Electrical stimulation (4 V, 1.0 Hz, 0.05-ms pulse width).

Fig. 8.

Representative examples of EUS activity and pelvic-EUS reflex after acute spinal cord transection at T8 in an animal with an empty bladder (A) and after distension of the bladder by injection of 0.2 ml of saline (B). *Top traces* were obtained without electrical stimulation, and *bottom traces* were obtained during stimulation. Reflexes were evoked by a single shock to the pelvic nerve. After bladder distension, reflexes evoked by the same electrical stimulation were recorded in the same animal. After drug administration, experimental trials were repeated before/after bladder distension. All recordings were obtained within 2–3 h after spinal cord transection. A1: EUS activity in the absence of electrical stimulation when the bladder was empty. A2 and A3: slight enhancement of ER and IR by 8-OH-DPAT (1.0

mg/kg iv) and suppression by WAY-100635 (1.0 mg/kg iv). *B1*: slight increase in EUS tonic activity in distended bladder. *B2*: enhancement of EUS tonic activity and LR by 8-OH-DPAT. *B3*: suppression of tonic EUS activity and LR, but no change in ER, by WAY-100635. Responses represent average of 10 individual reflexes recorded on a computer. ●, Electrical stimulation (6 V, 0.5 Hz, 0.05-ms pulse width).

Fig. 9.

Effect of WAY-100635 on facilitation of EUS EMG activity induced by bladder distension. Continuous recording was obtained from 1 spinal cord-intact animal. *A*: bladder distension by injection of 0.5 ml of air (▲) into the balloon or injection of 1.0 ml of saline (*) into the bladder. At beginning of bladder distension, EUS bursting activity was induced until the bladder was emptied (1). After the bladder was empty, EUS bursts disappeared, leaving only tonic activity (2). Bladder distension (air or saline) was repeated after WAY-100635 (0.1 or 0.4 mg/kg iv). After WAY-100635, duration of EUS bursting and tonic activity was reduced. Balloon was distended until EUS bursting and tonic activity disappeared. *B*: individual bursts at a faster sweep during time period in *A* indicated by 1. *C*: EUS tonic activity at a faster sweep during the time period in *A* indicated by 2.