Characterization of a spinal, urine storage reflex, inhibitory center and its regulation by 5-HT\textsubscript{1A} receptors in female cats

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Karicheti V, Langdale CL, Ukai M, Thor KB. Characterization of a spinal, urine storage reflex, inhibitory center and its regulation by 5-HT\textsubscript{1A} receptors in female cats. Am J Physiol Regul Integr Comp Physiol 298: R1198–R1208, 2010. First published February 17, 2010; doi:10.1152/ajpregu.00599.2009.—Urine storage is facilitated by somatic (pudendal nerve) and sympathetic [hypogastric nerve (HgN)] reflexes to the urethral rhabdosphincter (URS) and urethral smooth muscle, respectively, initiated by primary afferent fibers in the pelvic nerve (PelN). Inhibition of storage reflexes is required for normal voiding. This study characterizes a urine storage reflex inhibitory network that can be activated by PelN afferent fibers concurrently with the reflexes themselves. Electrical stimulation of PelN produced evoked potentials recorded by URS EMG electrodes (10-ms latency) or HgN electrodes (60-ms latency) in chloralose-anesthetized cats. When a second (i.e., paired) pulse of the same stimulus intensity was applied to the PelN 50–500 ms after the first, the reflexes evoked by the second stimulus were inhibited. The inhibition was maximal at paired-pulse intervals of 50–100 ms and remained after acute spinal transection at T10, confirming that the inhibitory center is located in the spinal cord. The 5-HT\textsubscript{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; 3–300 \mu g/kg iv) consistently reduced the paired-pulse inhibition from 20% to 60% of control in spinal-intact animals but had no effect in acute spinal animals (i.e., supraspinal site of action). N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-2-pyrindinylcyclohexanecarboxamide maleate (300 \mu g/kg iv) completely reversed 8-OH-DPAT's effects. The PelN-HgN reflex paired-pulse inhibition was not affected by 8-OH-DPAT. These results indicate the presence of a spinal, urine storage reflex, inhibitory center (SUSRIC) that is activated within 50 ms after activation of the reflexes themselves. SUSRIC is inhibited (disfacilitated) by supraspinal 5-HT\textsubscript{1A} receptors.

urethral rhabdosphincter; condition test stimulation; female feline; spinal inhibition

DURING URINE STORAGEx, SOMATIC NERVE ACTIVITY ORIGINATING IN MOTOR NEURONS OF ONUF'S NUCLEUS IS CARRIED ALONG THE PUDENDAL NERVE (PudN) TO THE URETHRAL RHABDOSPHINCTER (URS) AND CAUSES ITS CONTRACTION, THEREBY CREATING A HIGH-RESISTANCE BARREl TO INvOLUNTARY URINE RELEASE (26). SPHINCTER MOTOR NEURONS IN ONUF'S NUCLEUS CAN BE ACTIVATED BY SUPRASPINAL CENTERS VOLUNTARILY OR INVOLUNTARILY, AS DURING A COUGH, LAUGH, OR SNEEZE. IN ADDITION, THEY CAN BE ACTIVATED THROUGH A POLYSYNAPTIC, SACRAL SPINAL REFLEX PATHWAY FOLLOWING URETHRAL DISTENSION OR ELECTRICAL STIMULATION OF AFFERENT FIBERS TO THE PELVIC NERVE (PelN). CLINICALLY, THIS REFLEX IS REFERRED TO AS THE "GUARDING REFLEX" (26) AND IS ACTIVATED TO PREVENT URINE LEAKAGE DURING A COUGH, LAUGH, OR SNEEZE IF ABDOMINAL PRESSURE IS TRANSFERRED TO THE BLADDER AND URINE BEGINS TO OPEN THE BLADDER NECK. ELECTRICAL STIMULATION OF THESE AFFERENT FIBERS PRODUCES AN EVOKED POTENTIAL RECORDED FROM PudN EFFERENT FIBERS OR FROM THE URS AT A LATENCY OF ~10 MS (2, 10, 20, 23, 27).

IN ADDITION TO AN EXCITATORY COMPONENT OF PelN STIMULATION ON PudN EFFERENT PATHWAYS, A NUMBER OF INVESTIGATORS HAVE SUGGESTED AN INHIBITORY COMPONENT TO PelN STIMULATION ALSO EXISTS. FOR EXAMPLE, BRADLEY AND TEAGUE (2) SHOWED THAT A CONDITIONING STIMULUS APPLIED TO THE PelN 20–500 MS BEFORE STIMULATION OF AFFERENT FIBERS IN THE PudN, INHIBITED THE PudN-PelN REFLEX. SIMILARLY, McMAHON ET AL. (21) DESCRIBED A 50–1,000-Ms SUPPRESSION OF SPONTANEOUS ACTIVITY IN PudN EFFERENT FIBERS FOLLOWING ELECTRICAL STIMULATION OF THE PelN.

IN ADDITION TO THE ABOVE SOMATIC (I.E., PudN EFFERENT) STORAGE REFLEX, A SYMPATHETIC STORAGE REFLEX CAN ALSO BE RECORDED AS AN EVOKED POTENTIAL FROM THE HYPOGASTRIC NERVE (HgN) (4, 7, 19, 21) AT A LATENCY OF ~60 MS FOLLOWING PelN STIMULATION. THIS REFLEX IS THOUGHT TO MEDIATE CONTRACTION OF URETHRAL SMOOTH MUSCLE TO PROVIDE A TONIC INCREASE IN URETHRAL RESISTANCE AS THE BLADDER IS FILLED (26).

PHYSIOLOGICAL ACTIVATION OF THESE SOMATIC AND SYMPATHETIC REFLEXES IS SEEN DURING URODYNAMIC DISTENSION OF THE BLADDER AS IT SELLS WITH URINE AND CAN BE RECORDED AS ASYNCHRONOUS INCREASES IN HgN AND URS EMG ACTIVITY AND INCREASES IN URETHRAL PRESSURE (2, 7). HOWEVER, WHEN MICTURITION REFLEXES ARE ACTIVATED TO PRODUCE VOIDING, THERE IS A COORDINATED INHIBITION OF THE STORAGE REFLEXES. IN OTHER WORDS, WHEN THE PARASYMPATHETIC EFFERENT NEURONS ARE ACTIVE AND THE BLADDER CONTRACTS, THE URETHRA AND ITS SPHINCTER RELAXES THROUGH CENTRALLY MEDITATED INHIBITION OF THE SOMATIC (23) AND SYMPATHETIC (7) STORAGE REFLEXES.

THE PURPOSE OF THE PRESENT STUDY IS TO CHARACTERIZE PelN AFFERENT-MEDIATED MECHANISMS INVOLVED IN INHIBITION OF SOMATIC AND SYMPATHETIC OUTPUT TO THE URETHRA, WHICH ARE INDEPENDENT OF BLADDER PARASYMPATHETIC EFFERENT-MEDIATED MECHANISMS THAT ACCOMPANY BLADDER CONTRACTIONS. IN ADDITION, PREVIOUS STUDIES SHOWED THAT SEROTONERGIC AND NORADRENERGIC NEUROTRANSMITTER SYSTEMS ARE CLOSELY ASSOCIATED WITH CONTROL OF BOTH SOMATIC AND SYMPATHETIC STORAGE REFLEXES (4–6, 8, 10–15, 19, 26, 28, 29). IN PARTICULAR, A STRONG FACILITATION OF URS ACTIVITY WAS PRODUCED BY 5-HT\textsubscript{1A} RECEPTOR ACTIVATION (3, 9, 29).

THEREFORE, THE PRESENT STUDY FURTHER EXAMINED THE EFFECTS OF THE 5-HT\textsubscript{1A} RECEPTOR AGONIST, 8-HYDROXY-2-(DI-N-PROPYLAMINO)TETRALIN HYDROBROMIDE (8-OH-DPAT) AND THE 5-HT\textsubscript{1A} RECEPTOR ANTAGONIST N-2-[4-(2-METHOXYPHENYL)-1-PIPERAZINYL]ETHYL]-N-2-PYRIDINYL-CYCLOHEXANE-CARBOXAMIDE MALEATE (WAY-100635) ON SOMATIC AND SYMPATHETIC STORAGE REFLEXES USING IN VIVO ELECTROPHYSIOLOGY TECHNIQUES.

METHODS

All experiments were conducted under the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals and...
were approved by the Urogenix Institutional Animal Care and Use Committee.

Anesthesia was induced in 20 female cats (2.4 to 3.4 kg) with isoflurane and maintained with α-chloralose (65–100 mg/kg iv initially with 5 mg·kg$^{-1}$·h$^{-1}$ iv infusion throughout the experiment). A catheter was placed in the carotid artery to record arterial blood pressure. The trachea was also cannulated, and a probe was inserted to record expired CO$_2$ and respiration rate. Body temperature was

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**Fig. 1.** Top: frequency-response characteristics of the pelvic nerve-urethral rhabdosphincter (PelN-URS) reflex. Note obvious failure of evoked reflex potentials at frequencies ≥ 5 Hz. Bottom: 5-Hz stimulation at faster sweep speed. Note robust evoked PelN-URS reflex potential with 1st stimulus and failure of 2nd to 19th reflex potentials. Vertical calibration bar = 25 μV; horizontal calibration bar = 10 s in *top* trace and 0.33 s in *bottom* trace.

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**Fig. 2.** Condition-test (C-T), paired-pulse PelN-evoked potentials recorded from URS EMG electrodes (*top* traces) and hypogastric nerve (HgN) electrodes (*bottom* traces) at various interpulse intervals (A–F: each trace is the average of 10 sweeps). All traces are from a single animal. Note complete inhibition of test (T) URS- and HgN-evoked potentials at 50- and 100-ms intervals, partial recovery (arrows) at 200 and 500 ms, and return to control at 1,000 ms. C, conditioning pulse; T, test pulse. Thin vertical lines on traces are stimuli.
The spinal cord was cut at the T10–11 level (the most caudal vertebra that articulates with a rib) as a landmark, and the T10 vertebra was identified by using the T13 vertebra (the most cranial vertebra that articulates with a rib) as another landmark. The bladder was emptied as much as possible. In 11 cats, a laminectomy was performed around the 2nd lumbar vertebrae so that the mesenteric ganglion, and the distal end was cut and tied with saline-soaked 4-0 cotton suture for monopolar recording (Teflon-coated Pt/Ir wire, 10Ir7T). Recording electrodes were also placed in the urethra through the vaginal opening, and their position was verified by gross dissection after the experiment. The URS and HgN recording electrodes were connected to an AC amplifier (model PS11; Grass Instruments Division, Astro-Med, West Warwick, RI), and potentials were amplified ×10,000 and filtered (30 Hz to 3 kHz for URS and 3 Hz to 3 kHz for HgN signals). The PelN electrodes were connected to a stimulator (model S-88; Grass Instruments Division, Astro-Med) that was coupled to a stimulus isolation unit (model SI5; Grass Instruments Division, Astro-Med). The three different stimulation paradigms used are described in detail below. The data were acquired continuously using Chart (version 5.5.4), and evoked potentials were averaged (10 sweeps), stored, and analyzed using Scope (version 3.7.7) programs (ADIInstruments, Colorado Springs, CO).

Drugs were administered approximately every 30 min. 8-OH-DPAT (Sigma-Aldrich, St. Louis, MO) was dissolved in saline. WAY-100635 (Sigma-Aldrich) was dissolved in saline with gentle warming and sonication. All compounds were given intravenously through the jugular vein with a heating pad. A transurethral catheter (PE60) was placed into the urethra. The rectal temperature was recorded with an esophageal thermometer and maintained at 38–39°C ± 0.5°C.

Previous studies (2, 6) have shown (cf. Fig. 3b in Ref. 2) that evoked potentials recorded by the URS EMG electrodes in response to stimulation of the PelN with single (A) or paired (B and C) pulses. Notice that when the paired pulses were at 20-ms intervals (B) that the 2nd test stimulus produced an evoked potential that was similar in size to the 1st conditioning stimulus potential; but when the 2nd stimulus was 50 ms after the 1st, the test potential is markedly inhibited. Thus, refractoriness of the motor neuron action potential generator cannot explain the later inhibition. Also note that the beginning of the 2nd evoked potential overlaps with the end of the 1st evoked potential when the interval between stimuli is 20 ms. In most cases, the overlap was greater than the above example. Therefore, we did not try to quantify the amplitude of the test potentials evoked at a < 50-s interval after the conditioning stimuli.

Detailed methods of nerve stimulation and recording can be found in previous publications (5, 19). Briefly, following a laparotomy and removal of the intestines, the PelN trunk was isolated 2–3 cm caudal to the inferior mesenteric ganglion, and the distal end was cut and tied with saline-soaked 4-0 cotton suture for monopolar recording (Teflon-coated Pt/Ir wire, 10Ir7T; Sigmond Cohn, New York, NY). A 2-cm length of the HgN was isolated 2–3 cm caudal to the inferior mesenteric ganglion, and the distal end was cut and tied with saline-soaked 4-0 cotton suture for monopolar recording (Teflon-coated Pt/Ir wire, 10Ir7T). Recording electrodes were also placed in the urethra through the vaginal opening, and their position was verified by gross dissection after the experiment. The URS and HgN recording electrodes were connected to an AC amplifier (model PS11; Grass Instruments Division, Astro-Med, West Warwick, RI), and potentials were amplified ×10,000 and filtered (30 Hz to 3 kHz for URS and 3 Hz to 3 kHz for HgN signals). The PelN electrodes were connected to a stimulator (model S-88; Grass Instruments Division, Astro-Med) that was coupled to a stimulus isolation unit (model SI5; Grass Instruments Division, Astro-Med). The three different stimulation paradigms used are described in detail below. The data were acquired continuously using Chart (version 5.5.4), and evoked potentials were averaged (10 sweeps), stored, and analyzed using Scope (version 3.7.7) programs (ADIInstruments, Colorado Springs, CO).

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The URS was stimulated with square-wave pulses of 0.05-ms duration, 0.1–10.0 V (working voltage) at 0.5 Hz. The working stimulus voltage was set empirically at twice the threshold stimulus voltage (stimulation threshold for generating HgN-and URS-evoked potentials). The signals were quantified by measuring the rectified area under the curve (AUC). The AUC of PelN-HgN and PelN-URS reflexes were quantified for each drug dose response and normalized to the control response (mean ± SE). Evoked potentials were acquired at 5, 15, and 25 min after each drug dose, but since there were no significant differences, only responses at 5 min are shown. The effects of 8-OH-DPAT were evaluated using one-way repeated-measures (Friedman’s test) ANOVA. In addition, the Dunn’s multiple-comparison test was performed to compare the differences in

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**Fig. 3.** Absence of motor neuron refractoriness at 20-ms intervals. Evoked potentials recorded by the URS EMG electrodes in response to stimulation of the PelN with single (A) or paired (B and C) pulses. Notice that when the paired pulses were at 20-ms intervals (B) that the 2nd test stimulus produced an evoked potential that was similar in size to the 1st conditioning stimulus potential; but when the 2nd stimulus was 50 ms after the 1st, the test potential is markedly inhibited. Thus, refractoriness of the motor neuron action potential generator cannot explain the later inhibition. Also note that the beginning of the 2nd evoked potential overlaps with the end of the 1st evoked potential when the interval between stimuli is 20 ms. In most cases, the overlap was greater than the above example. Therefore, we did not try to quantify the amplitude of the test potentials evoked at a < 50-s interval after the conditioning stimuli.

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**Fig. 4.** Time dependence of C-T interval on paired-pulse inhibition. Note that the paired-pulse inhibition of the PelN-URS reflex lasted for about 1 s, while the paired-pulse inhibition of the PelN-HgN reflex lasted about 4 s. Also note a nonsignificant decrease in paired-pulse inhibition of PelN-URS reflex by acute T10 spinal cord transection (n = 9 for intact PelN-URS, n = 11 for PelN-URS spinalized; 2-way repeated-measures ANOVA comparing intact vs. spinal cord transected groups). PelN-HgN was also not affected by T10 spinalization (n = 4 for PelN-HgN reflex except for 4,000 ms, where n = 3 and 9 for PelN-HgN spinalized; 2-way repeated-measures ANOVA comparing intact vs. spinal cord transected groups between 200 to 500 ms C-T intervals). In addition, there was a significant difference in the magnitude of inhibition between 200 to 500 ms C-T intervals in the intact PelN-URS vs. PelN-HgN reflexes (2-way repeated-measures ANOVA, P < 0.05) and transected PelN-URS vs. PelN-HgN reflexes (2-way repeated-measures ANOVA, P < 0.05). Note that the 1,500 ms C-T interval for the PelN-HgN was applied every 5 s. The 3,000 and 4,000 ms C-T intervals were applied every 10 s. AUC, area under the curve.
the means of individual pairs when the ANOVA was significant. A Wilcoxon signed-rank t-test was also used to compare 300 μg/kg 8-OH-DPAT to WAY-100635 (Prism 5 for Windows, GraphPad Software, San Diego, CA). P < 0.05 was considered statistically significant.

**Condition test paradigm.** Condition test paradigms were applied every 2 s with a conditioning stimulus applied at intervals of 50, 100, 200, 300, 400, 500, and 1,000 ms prior to test stimuli, except as noted in Fig. 3. These “condition test (C-T)” or “paired pulse” evoked potentials were averaged and quantified as described above. The AUC of the test pulse was expressed as a percentage of the AUC of the condition pulse potential. The PelN-URS reflex comparing intact vs. T10 spinalization was analyzed by two-way repeated-measures ANOVA. The PelN-HgN reflex comparing intact vs. T10 spinalization between the condition intervals of 200 and 500 ms was analyzed by two-way repeated-measures ANOVA. The intact (PelN-URS vs. PelN-HgN) and T10 spinalization (PelN-URS vs. PelN-HgN) reflexes between the condition intervals of 200 and 500 ms were also analyzed by two-way repeated-measures ANOVA. In addition, the PelN-URS (intact and T10 spinalization) and PelN-HgN (intact only) reflexes comparing 8-OH-DPAT vs. control and 8-OH-DPAT vs. WAY-100635 were analyzed by two-way repeated-measures ANOVA. The Bonferroni posttest was then performed to compare the differences in the individual pairs of means when the ANOVA was significant. Finally, the dose response of 8-OH-DPAT at the condition interval of 100 ms was analyzed using one-way repeated-measures (Friedman’s test) ANOVA followed by Dunn’s multiple comparison test to compare individual pairs of means. P < 0.05 was considered statistically significant.

**Frequency response.** The PelN was continuously stimulated as above for 10 s at 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 50.0, and 100 Hz with 2 min between each frequency of stimulation. The mean URS EMG firing rate during the 10-s stimulation period (spikes/s) was measured using the Chart subroutine cyclic measurements/event count/simple threshold at a sampling rate of 4 kHz. The threshold for discriminating a spike was empirically set to twice the baseline voltage. The effects of drugs on the frequency response relationship were acquired 15 to 25 min after each drug dose. Two-way repeated-measures ANOVA

![Fig. 5. Frequency-response characteristics of PelN-URS reflex before (A) and after (B) 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT; 100 μg/kg iv). A: control, frequency-response characteristics of PelN-URS reflex. Note obvious failure of evoked reflex potentials at frequencies ≥ 5 Hz (top trace). Frequencies at 5-Hz stimulation are at faster sweep speed. Note marked reduction in 2nd-50th PelN-URS-evoked potentials compared with the 1st at 5 Hz (bottom trace). B: same experiment as in A after administration of 8-OH-DPAT (100 μg/kg iv). Note remarkable facilitation of evoked responses at 5–20 Hz (top trace). Also note insignificant effect on 1st evoked potential, but substantial facilitation of the 2nd-50th evoked potentials (bottom trace). Vertical calibration bar = 25 μV; horizontal calibration bar = 10 s in top trace and 0.33 s in bottom trace.]
was performed comparing 8-OH-DPAT vs. control, WAY-100635 vs. control, and 8-OH-DPAT vs. WAY-100635 in both the intact and spinal transected cats. Also, WAY-100635 (given as first drug) vs. control for intact cats only (since 8-OH-DPAT had no effects in spinal transected cats and 5-HT release should be blocked by spinal transection, so administration of a 5-HT receptor antagonist is unlikely to produce an effect) was analyzed by two-way repeated-measures ANOVA. The Bonferroni posttest was performed to compare the differences in the individual pairs of means at each frequency when the ANOVA was significant. \( P < 0.05 \) was considered statistically significant.

RESULTS

Characterization of the spinal, urine storage reflex, inhibitory center. Electrical stimulation of the PeLN evoked reflex potentials recorded from the URS and HgN (e.g., Figs. 1–6). As previously reported (2, 7), these reflexes showed a graded response to increasing intensities of stimulation. As shown in Figs. 1 and 6, increasing the frequency of stimulation of the PeLN increased efferent activity recorded from the URS up to a frequency of 5–20 Hz, at which point, higher frequencies reduced URS efferent activity. The decrease in activity was immediate, i.e., upon the second stimulus within the stimulation period (Fig. 1, bottom).

To determine whether the rapid drop in the second URS-evoked potential was due to a concomitant activation of inhibitory neurons, a condition test (C-T) paradigm was used. Fig. 2 shows representative tracings of averaged evoked potentials recorded from the URS and the HgN in response to 0.5-Hz stimulation of the PeLN as a single pulse (Fig. 2A) or as paired pulses applied at various intervals (Fig. 2, B–F). This figure and Figs. 3 and 4 show that a second stimulus, applied at 50 or 100 ms after the first, results in a remarkably diminished evoked potential, which gradually recovers to control levels within 1,000 ms for the URS-evoked potential and within 4,000 ms for the HgN-evoked potential in animals with an intact spinal cord. To determine whether the rapid drop in the second URS-evoked potential was due to intrinsic action potential refractoriness of the sphincter motor neurons, shorter time intervals were also examined in those animals in which the evoked potential durations were brief enough to clearly distinguish between the conditioning potential and the test potential at a shorter interval, as in Fig. 3. This figure shows that a second large test-evoked potential could be recorded with a 20-ms C-T interval, while in the same animal, a longer, 50 ms, C-T interval produces a remarkably diminished second test potential. Thus, refractoriness is not the cause of the diminished response to the second stimuli of a pair delivered at a 50-ms interval but, instead, is dependent on activation of an inhibitory circuit that requires between 20 and 50 ms to be activated. C-T stimuli intervals of 10 and 20 ms resulted in overlap of their respective evoked potentials, which made quantification of each individual evoked potential impossible. Therefore, we could only reliably quantify evoked potentials with \( \geq 50 \)-ms intervals. Also important is the finding that the C-T intervals that produced maximal inhibition, i.e., 50, 100, and 200 ms, matched the interstimulation interval for those frequencies of stimulation that show a prominent drop off during high-frequency stimulation, i.e., 20, 10, and 5 Hz, respectively, indicating a common mechanism.

In an attempt to discriminate PeLN afferent fibers that activate the URS and HgN reflexes from those PeLN afferent fibers that inhibit the URS and HgN reflexes, we carefully attempted to discriminate the electrical threshold and maximal voltages for activation of PeLN afferent fibers that activate the URS and HgN reflexes from the electrical threshold of those PeLN afferent fibers that inhibit the URS and HgN reflexes; but we could not. In other words, the threshold and maximum voltages for activating the evoked potentials were the same as the threshold and maximal voltages that subsequently inhibit the next evoked potential.

Fig. 6. Frequency-response curves for PeLN (PEL)-evoked firing recorded from the URS electrodes during control periods after 300 \( \mu g/kg \) 8-OH-DPAT and after 300 \( \mu g/kg \) \( N\)-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-2-pyrindinylcyclohexancarboxamide maleate (WAY-100635) in spinal intact (A, \( n = 4 \)) and spinal-transected (B, \( n = 6 \)) cats. Note that 8-OH-DPAT increased firing at higher frequencies of stimulation in a WAY-100635-sensitive manner only when the spinal cord was intact. \( *P < 0.05 \) 8-OH-DPAT is significantly different from WAY-100635 with Bonferroni posttest following a significant 2-way ANOVA in spinal cord-intact cats.
To grossly localize the inhibitory center, spinal cord lesions were placed at specific spinal cord levels. Figure 4 shows that spinalization at T10 produces only slight, nonsignificant decreases in the degree of inhibition for both the PelN-URS and PelN-HgN reflexes and a slight reduction in the duration of the inhibition of the PelN-HgN reflex. Similarly, L5 spinal transection did not alter the PelN-URS reflex nor its C-T inhibition. This indicates that the inhibitory substrate is likely in the same spinal segments as the reflex pathway itself. Transection of the spinal cord at the L5 level cuts the connections for the PelN-HgN reflex pathway since the afferent fibers enter at S1–S2 and the efferent fibers exit at L1–L2. Thus one cannot localize the spinal level of the inhibition using spinal lesions below the L1 level.

Effects of 5-HT1A receptor agonist 8-OH-DPAT and antagonist WAY-100635. Figs. 5 and 6 show that administration of the 5-HT1A receptor agonist 8-OH-DPAT significantly enhances the ability of the URS to follow high-frequency PelN stimulation in intact (Fig. 6A) but not spinal transected (Fig. 6B) animals. The enhancement of the URS activity by 8-OH-DPAT is greatest at high frequencies and least at low frequencies in spinal intact cats (Figs. 5 and 6A). The enhancement of URS activity by 8-OH-DPAT was reversed by the 5-HT1A receptor antagonist WAY-100635. Close examination of the frequency-response curve before and after WAY-100635 shows that WAY-100635 also produced its greatest effects at high frequencies of stimulation (Fig. 6A). The facilitatory effects of 8-OH-DPAT on the frequency-response curve were not seen after acute T10 spinal transection (Fig. 6B).

Two reasonable mechanisms by which 8-OH-DPAT might facilitate URS activity during high-frequency stimulation are a direct facilitation or a reduction in inhibition. To examine direct facilitation, we used low-frequency stimulation (i.e., 0.5 Hz or 2,000 ms between stimuli) to activate the URS, which should include virtually no inhibitory component since the C-T inhibition lasted < 1,000 ms. At this low frequency of stimulation, 8-OH-DPAT had weak and highly variable facilitatory effects on the PelN-URS reflex activation (Fig. 7) and no effect...
on the PelN-HgN reflex. Following acute spinal transection at T10, there were no significant effects of 8-OH-DPAT on either PelN-URS (133.4 ± 26.95% of control) or PelN-HgN (100.7 ± 13.84% of control) reflexes.

In contrast to the weak and variable effects of 8-OH-DPAT on the low-frequency, single-pulse, evoked PelN-URS reflex, 8-OH-DPAT produced very consistent and significant reductions in C-T inhibition produced by paired pulses (Figs. 8 and 9A) in a dose-dependent manner (Fig. 9D) that was reversed by the 5-HT1A receptor antagonist WAY-100635 (Figs. 8 and 9A). Importantly, 8-OH-DPAT had no effect on C-T paired-pulse inhibition of the PelN-URS reflex in animals with an acute spinal transection at T10 (Fig. 9B). Also, no effects of 8-OH-DPAT were observed on the PelN-HgN reflex (Figs. 8 and 9C).

To determine whether 5-HT1A receptors controlling PelN-URS and PelN-HgN reflexes were tonically activated by endogenous 5-HT, the 5-HT1A receptor antagonist WAY-100635 was administered as the first drug. As shown in Fig. 10A, WAY-100635 did reduce URS activity at higher frequencies of stimulation and reduced the amplitude of the PelN-URS- and PelN-HgN-evoked reflexes (Fig. 10B). However, WAY-100635 produced only insignificant increases in C-T paired-pulse inhibition (Fig. 10C).

DISCUSSION

Electrical stimulation of afferent fibers in the PelN at 0.5 Hz frequency reliably produced an evoked potential recorded by electrodes in the URS (PelN-URS reflex) at a latency of ~10 ms. When the frequency of the stimulation was increased > 5 Hz, the evoked reflex was greatly diminished in size, as previously reported by Bradley and Teague (2). Importantly, we noted that the inhibition of the reflex was maximally evident in the reflex evoked by the second stimulus of the high-frequency train. This immediate decrease in the second reflex suggested that the decrease in the evoked reflex was not due to run down of transmitters in the reflex pathway but was more likely due to a briefly delayed activation of an inhibitory center by the first stimulus that prevented the second stimulus from evoking a reflex discharge (Fig. 11). This suggestion was confirmed by using a C-T paired-pulse paradigm, where it was demonstrated that a conditioning stimulus, applied to the PelN 50–1,000 ms prior to a test stimulus, inhibited the URS reflex potential evoked by the test stimulus. The motor neuron membrane is not intrinsically refractory to action potential generation in response to the test stimuli, since potentials of equal or greater size could be recorded to test stimuli delivered at

Fig. 8. Paired pulse inhibition at 100 (A, C, and E) and 200-ms (B, D, and F) intervals during control period (A and B), after 8-OH-DPAT (C and D), and after WAY-100635 (E and F). Note that during the control period at 100 ms, the 2nd stimulus pulse produces virtually no PelN-URS reflex (single arrow), while at 200 ms a very small PelN-URS reflex (single arrow) can be seen. Similarly, only a small PelN-HgN reflex (double arrow) can be seen at 200 ms stimulus intervals. Note that 8-OH-DPAT (300 µg/kg iv) greatly augments the PelN-URS reflex (single arrows), resulting from the 2nd pulse at both 100- and 200-ms intervals. Also note, however, that the 2nd PelN-HgN reflex remains strongly inhibited (double arrow). Note that WAY-100635 (300 µg/kg iv) reverses the effect of 8-OH-DPAT on the PelN-URS reflex (single arrows) and has no effect on the PelN-HgN reflex (double arrow).
intervals of < 50 ms (i.e., 10–20 ms, Fig. 3). Furthermore, single-unit recordings from URS motor neurons have shown that they commonly fire at rates of 20 Hz (24).

Importantly, the PelN-HgN reflex showed a similar pattern of inhibition to paired-pulse stimulation as the PelN-URS reflex. In other words, the second PelN-HgN-evoked potential was inhibited by the first PelN stimulation. Because the latency of the PelN-HgN reflex has a latency of 60 ms and a duration up to 40 ms, it was difficult to quantify the AUC of the test-evoked potentials at 50 and 100 ms after the conditioning stimulus. However, qualitatively, no test potential could be seen superimposed on, or following, the conditioning potential at 50- and 100-ms intervals. At 200- to 300-ms intervals, the AUC of the test-evoked potential was only 20% of the conditioning-evoked potential AUC. This degree of inhibition was similar to that seen for the PelN-URS paired-pulse inhibition. However, the duration of the paired-pulse inhibition of the PelN-HgN reflex (4 s) was substantially longer than the duration of the PelN-URS paired-pulse inhibition (1 s).

The threshold stimulus intensity for evoking the inhibition of both the PelN-URS and the PelN-HgN reflexes was the same as the threshold stimulus intensity for evoking the reflexes, suggesting that the same, or at least similarly sized, primary afferent fibers activate both the somatic and sympathetic reflexes as well as their respective inhibitory centers.

The paired-pulse inhibition was slightly reduced (10–15%), but otherwise similar, after acute spinal transection at T10, indicating that the inhibitory circuit activated by the conditioning stimulus is located in the spinal cord. Inhibition of the PelN-URS reflex also remained after a spinal transection at the L5 level suggesting that the inhibitory circuit is located within the same segment as the PelN-URS reflex pathway (i.e., sacral cord). In regards to the spinal location of the inhibitory center, comparing firing rates across the frequency-response curves during control periods in spinal intact and transected animals (Fig. 6) suggests that spinalization may have enhanced the firing frequency during the control period. However, the differences between the firing rates in intact and spinal transected animals during control periods were not statistically significant.
Horseradish peroxidase tracing and c-fos activation studies have shown that primary afferent fibers in the PelN have spinal terminals that densely project to the dorsal gray commissure (DGC) region of the spinal cord from sacral to midlumbar levels (22, 30) and activate neurons in this region (32). Importantly, the DGC contains inhibitory GABAergic and glycineric neurons that, when electrically stimulated, inhibit URS activity (1, 25). Thus, inhibitory GABAergic and glycineric neurons that are found in the DGC might possibly be the cellular substrate of the spinal, urine storage reflex, inhibitory center (SUSRIC).

Importantly, the GABAergic and glycineric neurons in the DGC also receive input from the pontine micturition center and are also thought to be the cellular substrate for URS inhibition during micturition (1, 25). It is tempting to speculate that convergent inputs from primary afferent neurons on one hand, and descending supraspinal axons from the pontine micturition center on the other hand, provide strong coordination of URS inhibition for initiation of voiding as well as maintenance of URS inhibition until voiding is complete.

Administration of the 5-HT1A receptor agonist 8-OH-DPAT significantly reduced the paired-pulse inhibition from 20% of control to 60% of control in spinal intact animals. The 8-OH-DPAT-induced reduction in paired-pulse inhibition was reversed by WAY-100635, a highly selective 5-HT1A receptor antagonist, which confirms the role of 5-HT1A receptors in suppressing SUSRIC. 8-OH-DPAT also enhanced the ability of the PelN-URS reflex to follow high frequency (i.e., 2–10 Hz) stimulation. Importantly, 8-OH-DPAT reduced the inhibition in spinal cord-intact cats but not in animals with an acute T10 spinal transection, indicating that the relevant 5-HT1A receptors are located supraspinally.

8-OH-DPAT produced only weak and variable increases in the PelN-URS reflex evoked by low-frequency (e.g., 0.5 Hz)
stimulation. Furthermore, WAY-100635, administered as the first drug, had no effects on SUSRIC inhibition of the PeIN-URS reflexes. This suggests that neither SUSRIC nor 5-HT₁₆ receptor-sensitive descending input to SUSRIC are tonically active in chloralose-anesthetized male cats. Similarly, the fact that spinal transection produced only a slight, nonsignificant decrease in paired-pulse inhibition supports the suggestion that supraspinal-descending control of SUSRIC is not tonically active in chloralose-anesthetized cats. On the other hand, WAY-100635 administered as the first drug in intact animals did reduce the reflexes evoked by single-pulse stimulation (cf. Fig. 10, A and B). Possibly, there are additional 5-HT₁₆ receptor systems that are tonically active and provide weak facilitatory inputs to the storage reflexes. In contrast to the strong reduction in paired-pulse inhibition of the PeIN-URS reflex, 8-OH-DPAT had no effect on the paired-pulse inhibition of the PeIN-HgN reflex. This indicates an organizational difference between the sympathetic and somatic storage reflexes and allows for the existence of two separate SUSRICs, one for the sympathetic storage reflex and another for the somatic urinary storage reflex.

Previous urodynamic studies in anesthetized cats (17, 18, 29) support a supraspinal location of 5-HT₁₆ receptors that facilitate URS activity. This support stems from urodynamic studies that showed a robust, dose-dependent, and WAY-100635-sensitive enhancement of spontaneous URS EMG activity in chloralose-anesthetized cats under conditions of bladder irritation when the spinal cord is intact (29) but not when the spinal cord is transected, even after spinal bladder reflexes have emerged following spinal shock (17, 18). In other words, it is proposed that 8-OH-DPAT’s enhancement of URS activity during urodynamic studies results from stimulation of supraspinal 5-HT₁₆ receptors, which subsequently results in a reduction of SUSRIC-mediated inhibition of URS motor neurons, i.e., URS excitation results from disinhibition.

Figure 11 diagrams a model for PeIN-activated inhibition of urinary storage reflexes that incorporates the data from the present study. Activation of visceral primary afferent fibers contained in the PeIN reflexively activate somatic (to URS) and sympathetic (to HgN) pathways to the urethra. The same or similar PeIN fibers rapidly (i.e., <50 ms) and concomitantly activate inhibitory interneurons in the spinal cord that powerfully though briefly (i.e., 50–500 ms) suppress somatic and sympathetic efferent pathways. One might propose that this brief but powerful inhibition ensures that the sphincters are inhibited when PeIN primary afferent action potential frequency is high (i.e., >10 Hz), e.g., physiologically during micturition or pathologically when the bladder is overly distended.

Indeed, physiological correlates were demonstrated where increasing PeIN activity through gradual bladder distension inhibited the PeIN-URS reflex as well as the spontaneous URS EMG activity (2, 23). This inhibition is modest until bladder distension actually evoked a micturition contraction, at which point the inhibition is nearly complete. In regards to the paired-pulse inhibition of the PeIN-HgN, gradual distension of the bladder did not inhibit the PeIN-HgN reflex (7) even though these authors also found that the reflex was inhibited by paired-pulse stimulation similar to that of the present study and they found that during a micturition contraction evoked by bladder distension that the PeIN-HgN reflex was strongly inhibited. In relation to lower urinary tract dysfunction, underactivity of SUSRIC may be involved in bladder-sphincter dyssynergia and retentive urinary dysfunction such as Fowler’s syndrome (16), while overactivity of SUSRIC may be involved in the pathophysiology of stress urinary incontinence. A possible clinical correlation of SUSRIC activation may be the elegant demonstration in men that conditioning stimuli applied to the dorsal nerve of the penis (i.e., PudN afferent fibers) inhibited URS contractions reflexively evoked by magnetic stimulation of the spinal cord applied at intervals of 20–100 ms after the conditioning stimuli (31). Further examination of SUSRIC to characterize its physiological and pathological roles may provide another site for pharmacological therapy aimed at avoiding dysfunctions.

**Perspectives and Significance**

The present study describes a powerful spinal inhibitory mechanism activated by PeIN primary afferent fibers that suppresses urine storage reflexes to produce urethral relaxation, which we have termed SUSRIC. SUSRIC is independent of the inhibition of storage reflexes that accompanies bladder contractions and coordinated micturition. SUSRIC may serve to reinforce urethral relaxation during micturition, or it may serve as an emergency or backup system to promote urethral relaxation and urine release when the bladder becomes dangerously full but does not contract to produce coordinated micturition, e.g., following spinal cord damage. Insufficient SUSRIC activity may be involved in bladder-sphincter dyssynergia, while excessive activity may contribute to urinary incontinence.

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**DISCLOSURES**

No conflicts of interest are declared by the authors.

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