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Acute anal stretch inhibits NMDA-dependent pelvic-urethra reflex potentiation via spinal GABAergic inhibition in anesthetized rats

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Chen S-L, Huang Y-H, Kao Y-L, Chen G-D, Cheng C-L, Peng H-Y, Liao J-M, Huang P-C, Tsai S-J, Lin T-B. Acute anal stretch inhibits NMDA-dependent pelvic-urethra reflex potentiation via spinal GABAergic inhibition in anesthetized rats. *Am J Physiol Renal Physiol* 295: F923–F931, 2008. First published July 16, 2008; doi:10.1152/ajprenal.90254.2008.—The impact of acute anal stretch on the pelvic-urethra reflex potentiation was examined in urethane-anesthetized rats by recording the external urethra sphincter electromyogram activity evoked by the pelvic afferent stimulation. Test stimulation (1 stimulation/30 s) evoked a baseline reflex activity with a single action potential that was abolished by gallamine (5 mg/kg iv). On the other hand, the repetitive stimulation (1 stimulation/1 s) induced spinal reflex potentiation (SRP) that was attenuated by intrathecal 6-cyano-7-nitroquinoxaline-2,4-dione (a glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionat receptor antagonist, 100 μM, 10 μl) and D-2-amino-5-phosphonovalerate [a glutamatergic *N*-methyl-D-aspartate (NMDA) antagonist, 100 μM, 10 μl]. Acute anal stretch using a mosquito clamp with a distance of 4 mm exhibited no effect, whereas distances of 8 mm attenuated and 12 mm abolished the repetitive stimulation-induced SRP. Intrathecal NMDA (100 μM, 10 μl) reversed the abolition on SRP caused by anal stretch. On the other hand, pretreated bicuculline [γ-aminobutyric acid (GABA) A receptor antagonist, 100 μM, 10 μl] but not hydroxysaclofen ($GABA_B$ receptor antagonist) counteracted the abolition on the repetitive stimulation-induced SRP caused by the anal stretch. All of the results suggested that anal stretch may be used as an adjunct to assist voiding dysfunction in patients with overactive urethra sphincter and that GABAergic neurotransmission is important in the neural mechanisms underlying external urethra sphincter activity inhibited by anal stretch.

spinal cord; voiding dysfunction; glutamate; bicuculline; hydroxysaclofen

THE MECHANISM INVOLVED IN micturition, which is the result of coordination between bladder detrusor and outlet, is intricate. Both elements, the detrusor and outlet, maintain a sound cycle of storage and evacuation that is controlled by a group of reflex and voluntary actions (54). During the storage phase of a micturition cycle, detrusor relaxes and urethra contracts to produce continence; while the detrusor contracts, with sphincter relaxes to void urine during the evacuation phase. Inhibition of external urethra sphincter (EUS) activity during evacuation is essential for sufficient bladder emptying (18, 49). The absence of suppression on EUS activity during voiding is one feature of the pathological condition referred to as detrusor-sphincter dyssynergia (51). Such a dyssynergic sphincter contraction results in high intravesical pressure and residual urine. Therefore, to achieve near-normal voiding function in patients with detrusor-sphincter dyssynergia, outlet resistance should be reduced.

Arising from the puborectalis muscle, EUS innervated by the perineal branch of the pudendal nerve and external anal sphincter (EAS) innervated by the rectal branch of the pudendal nerve are both striated skeletal muscles that contract and relax voluntarily. A study investigating the coordination between the EUS and EAS muscles demonstrated these muscles shared in reflex actions as in dilatation and closing anal reflexes (53). It is interesting that inserting examining fingers in anus for anal stretch caused marked inhibition of the electromyogram (EMG) activity in both EUS and EAS (48). In able-bodied persons, EMG recording from the EUS and EAS during micturition and cystometry also showed simultaneous electric activity in these muscles (1, 50, 62). Results coming from clinical studies suggested anal stretch could be a useful technique to facilitate voiding in overactive urethra sphincter patients (15, 36, 38). However, the effects of anal stretch to EUS in normal individuals have not yet been established in the literature.

Cross-talk via the convergent neural pathways within the lumbosacral spinal cord is important for the normal regulation of sexual, bowel, and bladder functions (22, 45). Alterations in these convergent neural pathways cause a pathological mechanism by which injury or inflammation in one organ may lead to modifications in the function of other organs (4). In the pelvis, chemical and mechanical irritation in urethra may enhance the activity of not only striated urethra sphincter muscle itself but also EAS, implying that a neural-mediated cross-talk existed between external anal and urethra sphincter (60).

The pelvic-urethra reflex activity is presumed to be involved in the development of urethral resistance (14). Recent studies on pelvic-urethra reflex, using intact spinal cord preparations, have

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demonstrated a glutamatergic *N*-methyl-D-aspartate (NMDA)-dependent spinal reflex potentiation in which the activity of this reflex was dynamically potentiated by repetitive (29) and tetanic (30) afferent inputs. Because pathological potentiation in this reflex activity was suggested to underlie the hyperactive urethra (10, 11, 26–28), the activity-dependent spinal reflex potentiation seems to be a novel animal model for studying urethra continent function (12, 13, 28, 39, 41–44).

The identity of neurotransmitters responsible for EUS suppression during micturition, acting at receptors either on motoneurons, interneurons, or central sensory afferent terminals, is not well known (55). GABAergic terminals have been shown on EUS motoneuron cell bodies and dendrites of motoneurons within Onuf's nucleus (46). In both human studies (37, 56) and animal experiments (32, 33), administration of the γ-aminobutyric acid (GABA) agonist baclofen has been shown to decrease not only limb reflex output but also bladder and EUS activity. It has also been reported that intrathecal injection of GABAergic antagonists promotes the micturition reflex (21). All of these studies suggested GABAergic neurons are involved in the inhibitory pathway of EUS.

To shed light on the issue whether anus distension may relieve or attenuate detrusor-urethra sphincter dyssynergia by suppressing the urethra activity, we investigated the impact of anal stretch on the induction of spinal reflex potentiation in the pelvic-urethra reflex activity. In addition, the possible neurotransmitters involved were also studied to clarify the mechanisms underlying this phenomenon.

EXPERIMENTAL PROCEDURES

Animal Preparations

Adult female Wistar rats weighing 200–250 g were anesthetized using an intraperitoneal injection of urethane (1.2 g/kg). The animal care and the experimental protocol were in accordance with the guidelines of the National Science Council of Taiwan and the guidelines of the National Institutes of Health's Care and Use of Laboratory Animals (NIH Publications No. 80–23) revised in 1996. All efforts were made to minimize both animal suffering and the number of animals used throughout this study. This study was reviewed and approved by the Institutional Review Board of Chung-Shan Medical University in Taichung, Taiwan. The trachea of the animal was intubated to keep the airway clear. A PE-50 catheter (Portex; Hythe, Kent, UK) was placed in the left femoral vein for administration of anesthetics when needed. A midline abdominal incision was made to expose the pelvic viscera. A wide-bore bladder cannula (PE-50) was inserted in the lumen of the urinary bladder from the apex of the bladder dome and was secured with cotton thread. The right pelvic nerve was dissected carefully from the surrounding tissues and was then transected as distally as possible for stimulations, whereas the left pelvic nerve was left intact. In experiments exploring the effect of anal stretch on the pelvic-urethra reflex activity, the end of the cannula was left open to the air and drained freely (Fig. 1*A*) to avoid urine accumulation in the bladder during experiment, which may alter the reflex activity. In cystometry experiments, after the trigone was ligated, the intravesical catheter was connected to a volume reservoir and a pressure transducer to test the effect of anal stretch on the urethra activity under bladder distension with both pelvic nerves intact (see Fig. 5*A*). The rats were monitored for the corneal reflex and a response to noxious stimulation to the paw throughout the experiment. If either was present, a supplementary dose of anesthetics (0.4 g/kg urethane) was given through the venous catheter. At the end of the experiment, the animals were killed by an intravenous injection of potassium chloride saturation solution under deep anesthesia.

Intrathecal Catheter

The occipital crest of the skull was exposed, and the atlanto-occipital membrane was incised at the midline with the tip of an 18-gauge needle. A PE-10 catheter was inserted through the slit and passed caudally to the dorsal arachnoid space at the T_{13} vertebrae level (which is the level at the L_5-S_2 spinal cord). The volume of fluid within the cannula was kept constant at 10 μl in all experiments. A single, 10-μl volume of drug solution was administered followed by a 10-μl flush of vehicle solution. At the end of each experiment, the location of the injection site was marked by an injection of Alcian blue (10 μl, 2%), and a laminectomy was performed to verify the location of the cannula tip. The volume of drug injected in the spinal cord in this experiment has been reported to spread from 0.5 to 1.5 mm from the site of injection as described previously (12). The data obtained from animals whose cannula tip deviated by >0.5 mm from the upper and lower limits of the dorsal aspect of the arachnoid space along L_5 to S_2 were excluded from the statistical analysis.

EMG Recordings

Epoxy-coated copper wire (50 μm; Giken, Tokyo, Japan) EMG electrodes were placed into the periurethra area intra-abdominally using a 30-gauge needle with a hooked EMG electrode positioned at the tip. The needle was inserted in the sphincter ~1–2 mm lateral to the urethra and then was withdrawn, leaving the EMG wires embedded in the muscle. The EMG activity was amplified 20,000-fold, filtered (high-frequency cut-off at 3,000 Hz and low at 30 Hz) by a preamplifier (model 7P1; Grass, Cleveland, OH), and then continuously displayed on an oscilloscope (TDS 3014; Tectronics, Wilsonville, OR) and the recording system (MP30; Biopac, Santa Barbara, CA). The dissected nerve and the stimulating/recording electrodes were bathed in a pool of warm paraffin oil (37°C) to prevent drying.

Application of Drugs

Drugs dissolved in artificial cerebrospinal fluid with pH adjusted to 7.4 were used for intrathecal injections. Drugs used in the experiment were gallamine triethiodide (GL, 5 mg/kg), 6-cyano-7-nitroquinoxaline-2,4-dione [CNQX, a glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionat receptor antagonist; 100 μM, 10 μl; Sigma], D-2-amino-5-phosphonovalerate (APV, a glutamatergic NMDA receptor antagonist; 100 μM, 10 μl; Sigma), L-glutamate (100 μM, 10 μl; Sigma), NMDA (100 μM, 10 μl; Sigma), bicuculline [$GABA_A$ receptor antagonist; 100 μM, 10 μl; Sigma], and hydroxysacrofen (a $GABA_B$ receptor antagonist; 100 μM, 10 μl; Sigma). A solution of identical volume to the tested agents was dispensed intrathecally to serve as a vehicle. At the end of the experiment, the location of the injection site was marked by an injection of Alcian blue (2%, 10 μl). The volume of drug injection in the spinal cord in such experiment was reported to spread 0.5–1.5 mm from the site of injection (12). Therefore, a cannula positioned >0.5 mm away from the intended site of injection was not included in the statistical analysis.

Experimental Arrangement

Recording the numbers of action potentials evoked by the electric shocks assessed the pelvic-urethra reflex activity. The schematic arrangement of external urethra sphincter electromyogram (EUSE) recording and the pelvic afferent nerve fiber stimulation are shown in Fig. 1*A*. In the beginning of all experiments, we manipulated the stimulation intensity, and an electric intensity that caused a single spike action potential in the reflex activity was used to standardize the baseline reflex activity. This intensity was then used for stimulation throughout each experiment. The protocol for assessing the effects of electrical stimulation and different kinds of reagent/maneuver on the reflex activity was as follows.

Protocol 1: Pelvic afferent nerve test stimulation. Single shock at a fixed suprathreshold strength was repeated at 30-s intervals (1 stimulation/30 s) and given through a pair of stimulation electrodes for 30 min.

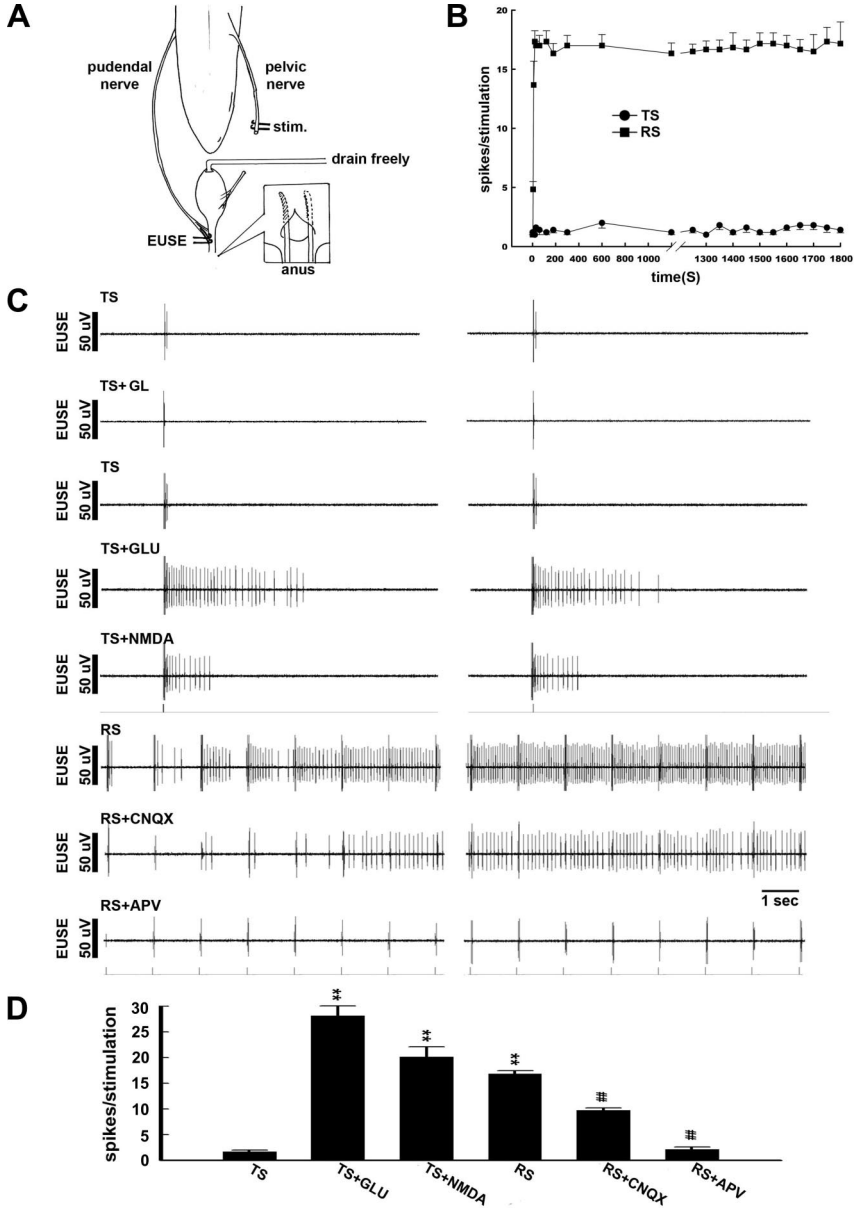

Fig. 1. Baseline pelvic-urethra reflex activity and reflex potentiation. *A*: experimental arrangements showing the external urethra sphincter electromyogram (EUSE) activity was recorded under the urinary bladder as it drains freely. *B*: summarized data showing the mean spike number evoked by each pulse of the test stimulation (TS) varied little over the stimulation period, whereas that evoked by the repetitive stimulation (RS) increased shortly following stimulation onset, reached a rather constant level, and maintained at that level until the cessation of stimulation (**$P < 0.01$ to TS, $n = 35$ animals). *C*: single pulses of pelvic afferent nerve TS (1 stimulation/30 s, indicated by the marks at the bottom) evoked a baseline reflex activity with single action potentials that was abolished by neuromuscular blockage using gallamine (TS + GL). In animals that received no gallamine injection, TS evoked a baseline reflex activity, whereas RS (1 stimulation/1 s, indicated by the marks at the bottom) gradually induced reflex potentiation in the EUSE activity. Intrathecal glutamate (TS + GLU) and *N*-methyl-D-aspartate (NMDA; TS + NMDA) both exhibited excitation on the TS-elicited reflex activities. Moreover, intrathecal 6-cyano-7-nitroquinoxaline-2,4-dione (CNQX) and D-2-amino-5-phosphonovalerate (APV) both exhibited inhibition on the RS-induced reflex potentiation. *D*: summarized data showing the mean spike number averaged at 30 min following stimulation onset evoked by TS and TS with intrathecal application of glutamate (TS + GLU) or NMDA (TS + NMDA) as well as RS and RS with intrathecal CNQX (RS + CNQX) or APV (RS + APV). **$P < 0.01$ to TS, ##$P < 0.01$ to RS, $n = 7$.

This frequency of stimulation was chosen for sampling data because it did not result in response facilitation.

Protocol 2: Pelvic afferent nerve repetitive stimulation. After a baseline period (usually 30 min), the repetitive stimulation (1 stimulation/1 s, lasting for 30 min) with intensity identical to the test stimulation was applied to induce reflex potentiation.

Protocol 3: Glutamatergic agonists/antagonists. After the equilibrium (usually 30 min), the glutamatergic agonist glutamate or NMDA was injected intrathecally 1 min before test stimulation onset to test their effects. In the experiment, the effects of the glutamatergic antagonist CNQX or APV injected intrathecally 1 min before repetitive stimulation onset were determined.

Protocol 4: Anal stretch. After a reflex potentiation has been established by the repetitive stimulation, anal stretch was carried out at 20 min following stimulation onset and maintained. Because balloon distension may cause vertical displacement that interferes the recording electrodes located in the urethra, we stretched the anus horizontally using a mosquito clamp, the tip of which was inserted in the anus for ~2 cm. The distances used for anal stretch were 4, 8, or 12 mm.

Protocol 5: GABAergic antagonists. After the equilibrium (usually 30 min), the $GABA_A$ or $GABA_B$ antagonist bicuculline or hydroxysacrofen was injected intrathecally 1 min before repetitive stimulation onset. Next, the repetitive stimulation associated with anal stretch was tested as in *protocol 4*.

Statistics

All data in the text and Figs. 1–5 are mean values ± SE. Statistical analysis of the data was performed by means of ANOVA. In all cases, a P value of <0.05 was considered to indicate statistical significance.

RESULTS

Baseline Reflex Activity and Reflex Potentiation

As shown in Fig. 1*C*, a single pulse of pelvic afferent nerve test simulation (1 stimulation/30 s) evoked a stable baseline reflex activity with single action potential in the EUSE activity.

In three animals, gallamine (5 mg/kg) was administrated intravenously after we connected these animals to a ventilator. Gallamine injection abolished the reflex activity evoked by the test stimulation. On the other hand, in animals that received no gallamine injection, repetitive stimulation (1 stimulation/1 s) induced reflex potentiation in the EMG (EUSE) activity in contrast to the test stimulation evoked in the baseline reflex activity. The summarized data in Fig. 1*B* show that the mean spike number evoked by the test stimulation varied little over the stimulation period ($P > 0.05$, $n = 35$), whereas that done by the repetitive stimulation increased shortly following onset of stimulation, reached a rather constant level, and maintained at that level until the cessation of stimulation. Moreover, the mean spike numbers evoked by the repetitive stimulation were significantly higher than that done by the test stimulation ($P < 0.01$, $n = 7$).

Glutamatergic Agonists and Antagonists

As shown in Fig. 1*C*, test stimulation on the pelvic afferent nerve evoked a baseline reflex activity with single action potential. Intrathecal pretreatments of glutamate (100 μM, 10 μl) and NMDA (100 μM, 10 μl) both induced a longer-lasting reflex potentiation. On the other hand, repetitive stimulation produced a long-lasting potentiation in the reflex activity. Intrathecal pretreatments of CNQX (100 μM, 10 μl) and APV (100 μM, 10 μl) both blocked the repetitive stimulation-induced reflex potentiation. The summarized data in Fig. 1*D* shows that pretreatments of glutamate and NMDA significantly increased the mean spike numbers evoked by the test stimulation averaged within 1 min counted at 30 min following stimulation onset (test stimulation, $P < 0.01$ to test stimulation, $n = 7$). Moreover, pretreatment of CNQX and APV significantly de-

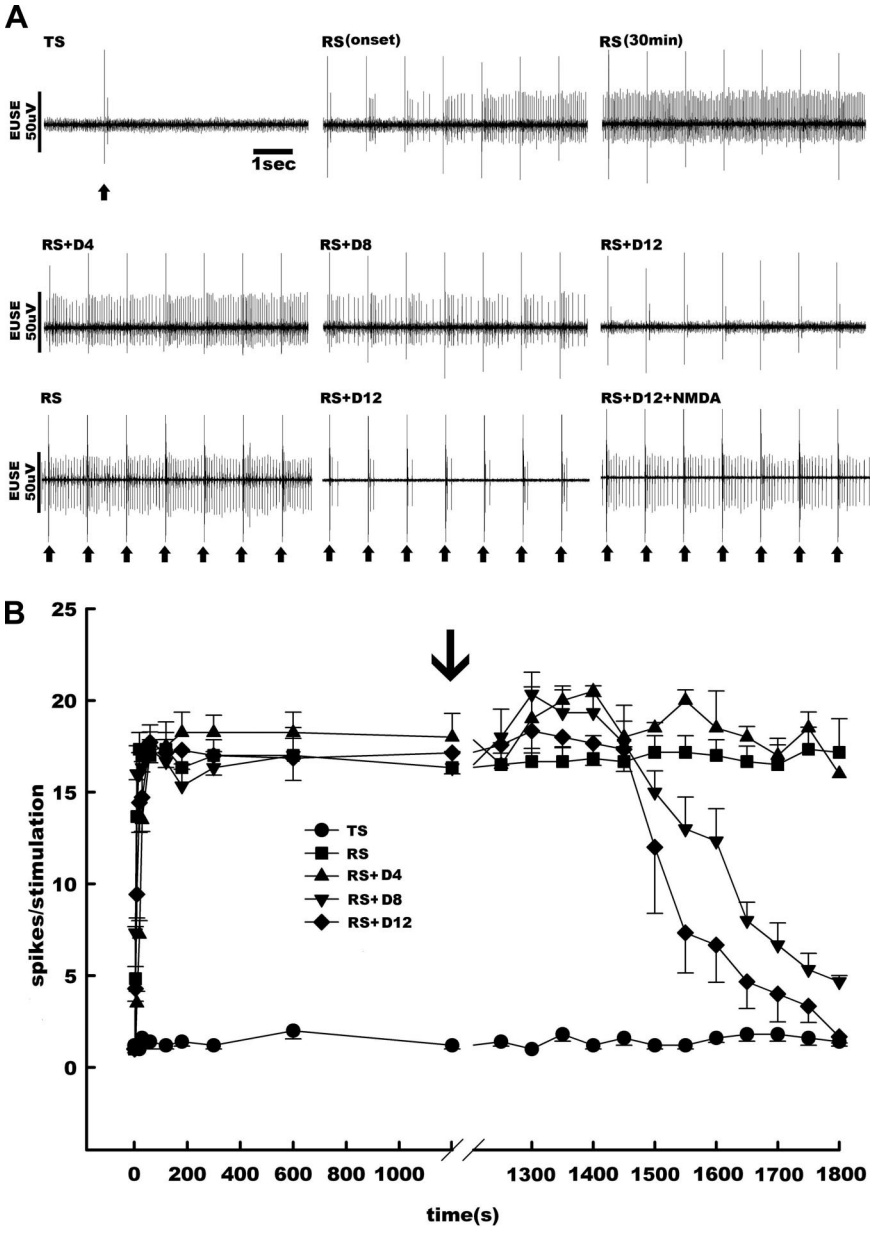

Fig. 2. Anal stretch inhibited the RS-induced reflex potentiation. *A*: single action potentials in the EUSE were evoked by the TS (1 stimulation/30 s, indicated by the arrow on *bottom*) at the pelvic afferent nerve, whereas a longer-lasting reflex potentiation was induced by RS (1 stimulation/1 s, indicated by the arrows on *bottom*). Onset and 30 min indicate the reflex activity at the first 6 s and 30 min following stimulation onset. Anal stretch with a distance of 4 mm (RS + D4) failed to affect while with distances of 8 mm (RS + D8) attenuated and of 12 mm (RS + D12) abolished the RS-induced reflex potentiation. Intrathecal NMDA (RS + D12 + NMDA) reversed the abolition on reflex potentiation caused by anal stretch with a distance of 12 mm (RS + D12 + NMDA). *B*: summarized data showing the mean spike numbers evoked by the TS, RS, RS + D4, RS + D8, and RS + D12. Acute anal stretch with distances wider than 8 mm significantly inhibited the RS-induced reflex potentiation (##$P < 0.01$ to RS, $n = 7$).

creased the mean spike numbers evoked by the repetitive stimulation ($P < 0.01$ to repetitive stimulation, $n = 7$).

Effects of Anal Stretch

As shown in Fig. 2*A*, after a reflex potentiation has been established by the repetitive stimulation, anal distension at 20 min following stimulation onset with a distance of 4 mm had no effect, whereas that with a distance of 8 mm attenuated the repetitive stimulation-induced reflex potentiation. Moreover, the established reflex potentiation was completely abolished by anal stretch with a distance of 12 mm. Mean spike number evoked by repetitive stimulation with anal stretch with distances of 4, 8, and 12 mm was summarized in Fig. 2*B*. The stepwise increment of the distance of anal stretch attenuated and eventually abolished the repetitive stimulation-induced reflex potentiation when the distances of anal stretch were wider than 8 mm ($P < 0.05$, $n = 7$).

NMDA Agonist

As shown in Fig. 2*A*, repetitive stimulation induced reflex potentiation in the EMG activity that is abolished by anal stretch with a distance of 12 mm. After the repetitive stimulation-induced reflex potentiation has been abolished, intrathecal NMDA injection reversed such an abolition caused by anal stretch.

GABAergic Antagonists

As shown in Figs. 3*A* and 4A, the repetitive stimulation-induced reflex potentiation was abolished by anal stretch with a distance of 12 mm; intrathecal pretreatment of hydroxy saclofen, a $GABA_B$ receptor antagonist, exhibited no effect on the abolition caused by anal stretch (Fig. 4*A*). On the other hand, as shown in Fig. 3*A*, pretreatment with bicuculline, a $GABA_A$ receptor antagonist, partly restored the reflex potentiation. The summarized data in Figs. 3*B* and 4*B* show bicuculline ($P < 0.05$, $n = 7$) but not hydroxysaclofen ($P > 0.05$, $n = 7$) reversed the attenuation in the mean spike number caused by anal stretch with a distance of 12 mm.

Cystometric Investigation

In three animals, after a ligation was made at the bladder trigone, the urinary bladder was connected to a volume reser-

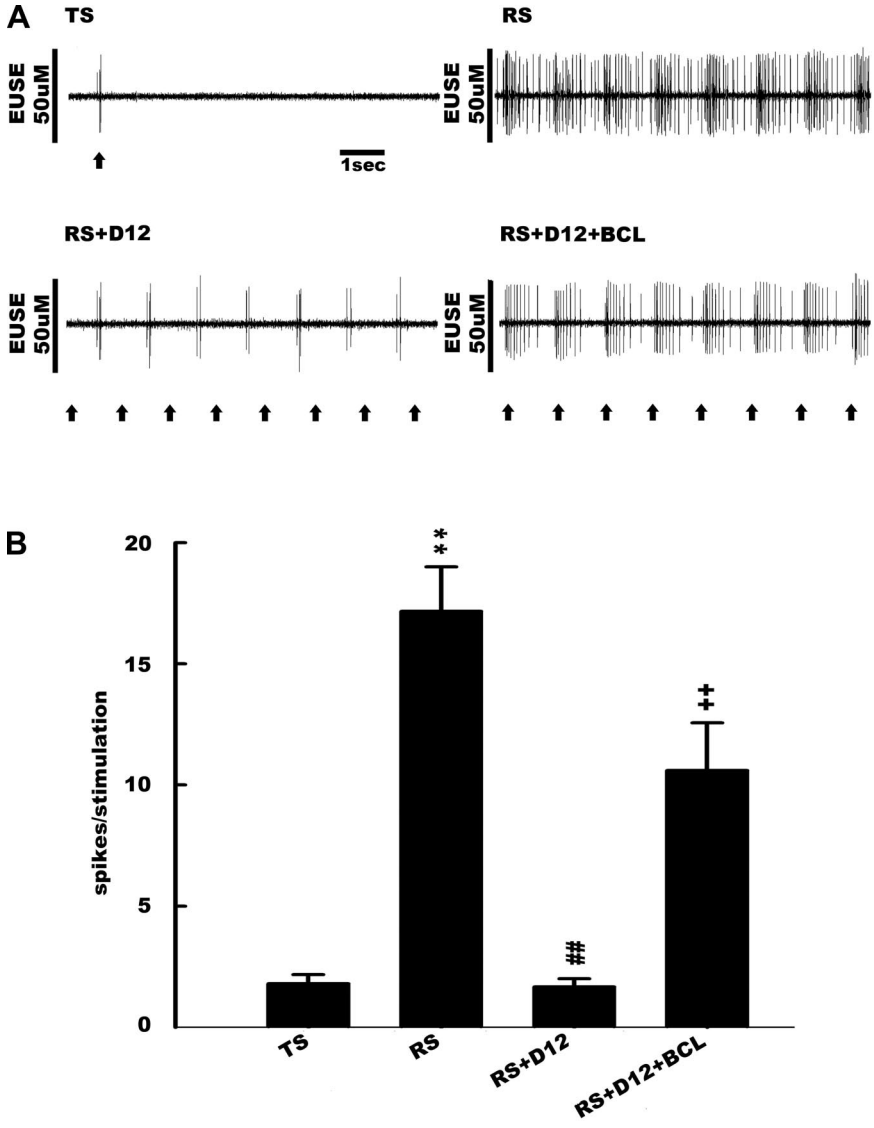

Fig. 3. Bicuculline, a $GABA_A$ antagonist, reversed the inhibition on the RS-induced reflex potentiation caused by anal stretch. *A*: pelvic afferent nerve TS (1 stimulation/30 s, indicated by the arrow on *bottom*) evoked a single action potential, whereas RS (1 stimulation/1 s, indicated by the arrows on *bottom*) induced a long-lasting reflex potentiation in the EUSE activity that was abolished by RS + D12. Moreover, intrathecal bicuculline pretreatment reversed the inhibition exhibited by anal stretch (RS + D12 + BCL). *B*: summarized data show the mean spike number evoked by each pulse averaged with 1 min at 30 min following stimulation onset, elicited by the TS, RS, RS + D12, and RS + D12 + BCL. $**P < 0.01$ to TS, $\#\#P < 0.01$ to RS, and $++P < 0.01$ to RS + D12, $n = 7$.

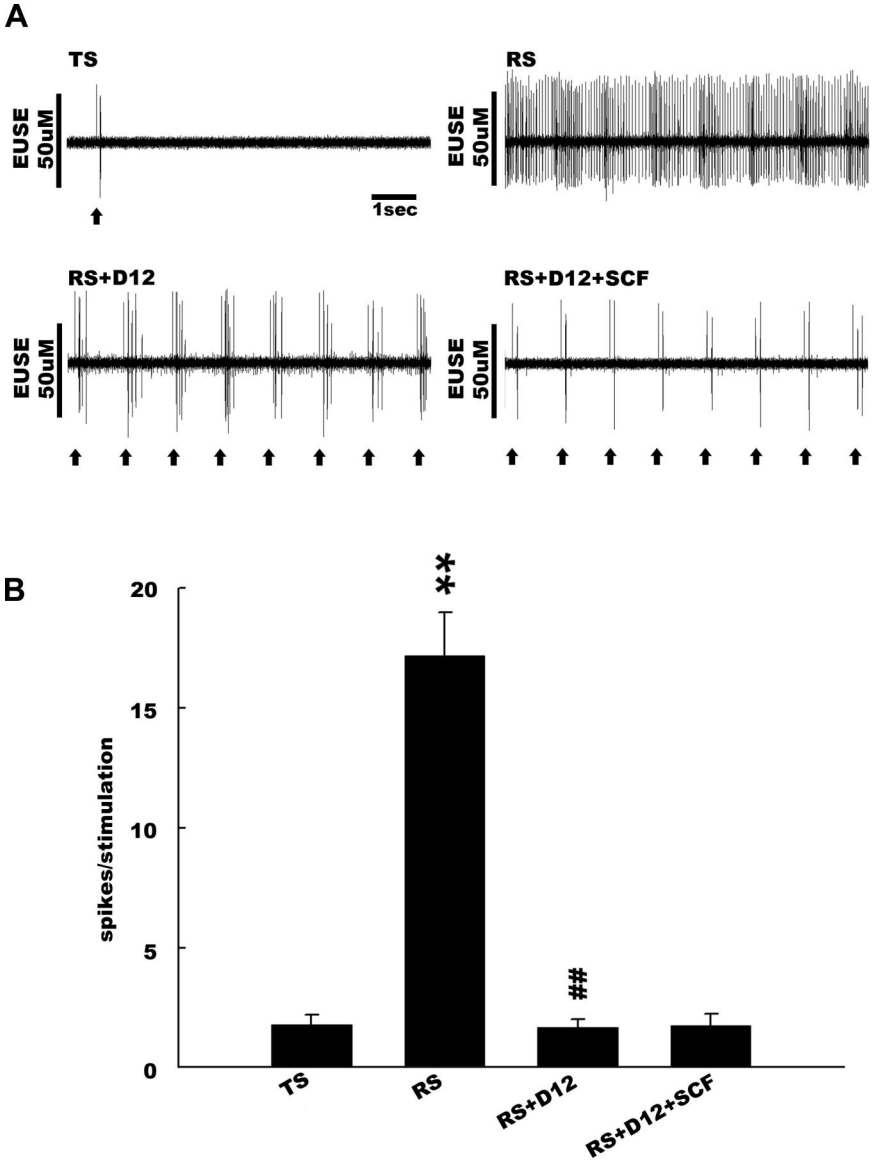

Fig. 4. Hydroxysacrofen, an $GABA_B$ antagonist, failed to reverse the inhibition on the RS-induced reflex potentiation caused by anal stretch. *A*: pelvic afferent nerve TS (1 stimulation/30 s, indicated by the arrow on *bottom*) evoked a single action potential, whereas RS (1 stimulation/1 s, indicated by the arrows on *bottom*) induced a long-lasting reflex potentiation in the EUSE activity that was abolished by RS + D12. Intrathecal hydroxysaclofen pretreatment failed to reverse the inhibition exhibited by anal stretch (RS + D12 + SCF). *B*: summarized data showing the mean spike number evoked by each pulse averaged within 1 min at 30 min following stimulation onset caused by the TS, RS, RS + D12, and RS + D12 + SCF. $**P < 0.01$ to TS, $\#\#P < 0.01$ to RS, and $++P < 0.01$ to RS + D12, $n = 7$.

voir and a pressure transducer (Fig. 5*A*) as described elsewhere (26). As shown in Fig. 5*A*, no spontaneous firing was recorded in the EMG activity when the pressure reservoir was positioned at the level identical to the urinary bladder (control). We then elevated and held the volume reservoir at the level 16 cm higher than the urinary bladder, and this maneuver caused firing in the EMG activity. Anal stretch with a distance of 12 mm attenuated and eventually abolished the firing caused by bladder distension.

DISCUSSION

In this in vivo animal study, we make the first direct demonstration that acute anal stretch may abolish NMDA-dependent repetitive stimulation-induced pelvic-urethra reflex potentiation, which is presumed to be involved in the development of urethra hyperactivity. In addition, pharmacological tests showed that intrathecal injection of low-dose bicuculline and NMDA both counteracted the abolition on the reflex potentiation caused by the anal stretch. These data suggest acute anal stretch may reflexively inhibit NMDA-dependent reflex potentiation via $GABA_A$ergic neurotransmssion at the spinal cord level.

GABA, which is readily accepted as a vital inhibitory neurotransmitter (7), exhibits presynaptic or postsynaptic inhibition on the primary afferent fibers at the spinal cord level (2, 3, 17). GABA elicits inhibitory effects on the superficial dorsal horn neurons through activation of the chloride-permeable $GABA_A$ receptors (61) or G protein-coupled $GABA_B$ receptors (6). GABA has also been identified as a critical inhibitory neurotransmitter for the spinal micturition circuitry and exerts its effect via activating either $GABA_A$ or $GABA_B$ receptors (21, 32, 63). GABAergic neurotransmission is known to have an inhibitory action on urethral function via effects on motor neurons to the urethra sphincter. When evacuation takes place, impulses descending from the pontine micturition center inhibit the motor neurons innervating the urethra sphincter via projections to the GABAergic premotor interneurons of Onuf's nucleus (5).

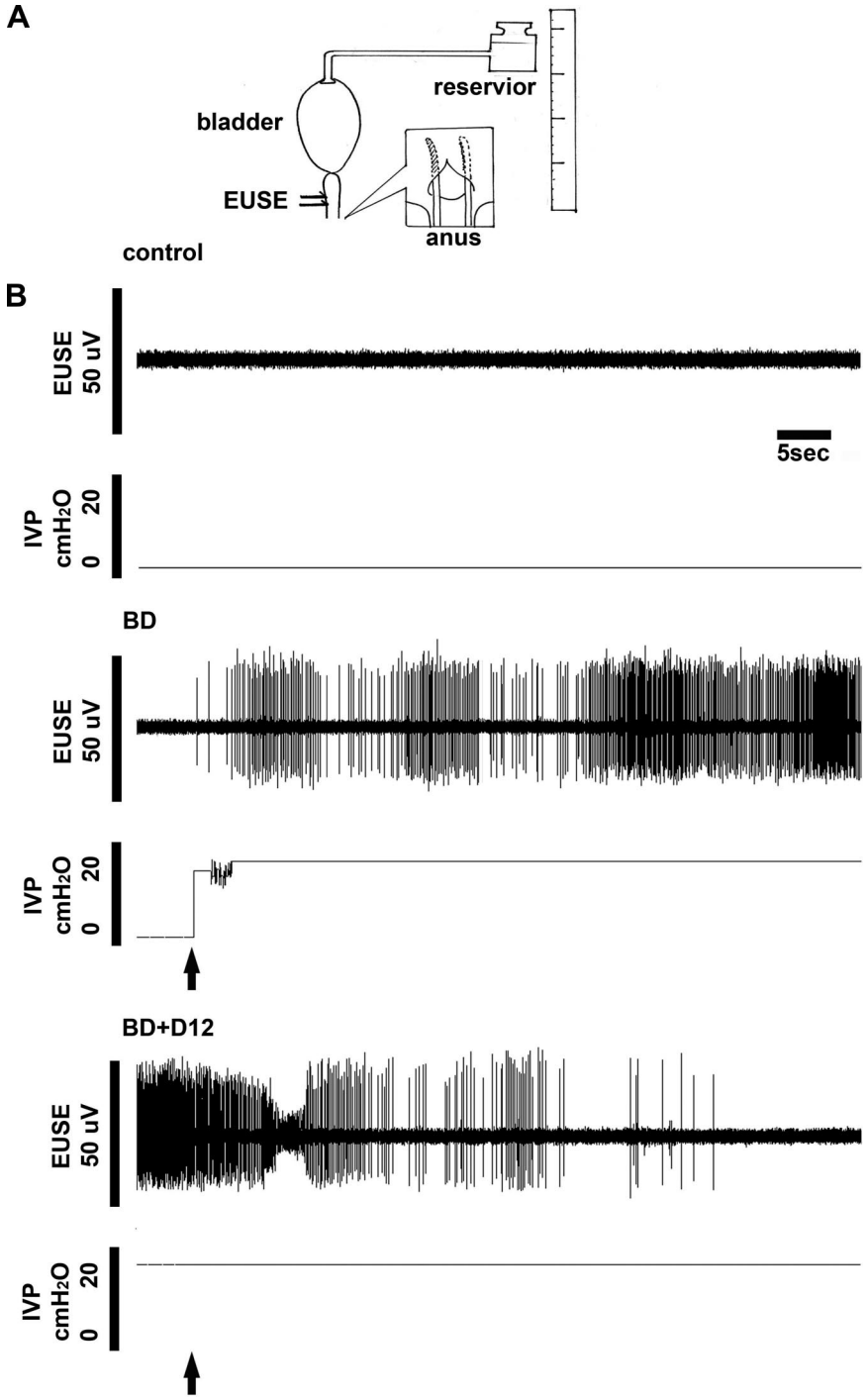

Fig. 5. Anal stretch reverses the urethra electromyogram activity caused by bladder distension. *A*: schematic arrangements showing the EUSE activity was recorded under the urinary bladder distension using a volume reservoir connected to the bladder via the bladder catheter. *B*: when the pressure reservoir was positioned at the level identical to the urinary bladder, there was no spontaneous firing recorded in the electromyogram activity (control). Elevating and then maintaining the volume reservoir at the level 16 cm higher than the urinary bladder caused firing in the electromyogram activity (BD, arrow indicates initial bladder distention) that was attenuated and eventually abolished the firing caused by bladder distension (BD + D12, arrow indicates initial anal stretch).

Not only the spinal integrated reflex activity but GABAergic neurotransmission may also affect activity-dependent reflex potentiation. In studies investigating long-term potentiation, a well-known form of activity-dependent reflex potentiation, GABAergic presynaptic and postsynaptic inhibitions were observed in rat hippocampal neurons (19). It is well established that activation of GABA receptor activity may attenuate or abolish the NMDA-dependent reflex potentiation via presynaptic or postsynaptic inhibition on the glutamatergic NMDA receptors (8). All of these results were quite correlated with the present study in which pretreatment with the $GABA_A$ receptor antagonist bicuculline reversed the attenuation on the induction of NMDA-dependent spinal reflex potentiation, indicating spinal $GABA_A$ergic neural inhibition plays a role in the abolition of the induction of reflex potentiation caused by anal stretch. However, despite injecting GABA antagonist intrathecally with a volume of 10 μl, the possibility of such a low dose of test agents exhibiting effects on the higher brain center was minor. Because we did not transect the spinal cord in this study, the possibility of descending modulation coming from a higher neurological center to inhibit spinal pelvic-urethra reflex potentiation cannot be ruled out.

However, in contrast to bicuculline, hydroxysaclofen, the $GABA_B$ receptor antagonist used in this study, appeared to have minimal effect on the reversal of the abolition on reflex potentiation caused by anal stretch. There are several possible causes that may be taken into account. First, $GABA_B$ antagonists have limited efficacy in modulating of synaptic transmission compared with $GABA_A$. It has been reported that application of weak $GABA_B$ antagonists, such as hydroxysaclofen, at the soma did not reach the distal synapse of neurons (57). In addition, postsynaptic $GABA_B$ receptors were less important in the regulation of motor neuron activity. Application of the $GABA_B$ agonist baclofen at a concentration sufficient to depress synaptic activity was not associated with changes in membrane potential, conductance, and excitability in the spinal motor neurons (16, 23, 25). Another possibility is that $GABA_B$ receptors on the afferent terminals are located extrasynaptically. Under such a condition, activation of extrasynaptic receptor is only likely to occur during periods of massive GABA release or reduced reuptake (57). Moreover, $GABA_B$ receptor may require longer exposure or a higher concentration of GABA for activation than $GABA_A$ receptors (40). Furthermore, hydroxysaclofen, the $GABA_B$ receptor antagonist, is a weak antagonist with possible agonistic properties (47). Therefore, clarification of the $GABA_B$ergic mechanism involved in anal stretch requires further investigation.

Arising from the puborectalis muscle, EUS innervated by the perineal branch of the pudendal nerve and EAS innervated by the rectal branch of the pudendal nerve are both striated muscles. In addition to voluntarily contracting or relaxing while the other does not, both of these muscles reflexively contract or relax simultaneously (53). Electophysiological evidence demonstrated that basal activity of the external urethral sphincter was altered by electric stimulation of the EAS in healthy volunteers (52). In addition, vigorous distension of anal sphincter led to inhibition of urethra and anal sphincter activity in most spinal cord-injured patients (48). Furthermore, a recent study investigating urodynamic responses to anal stretch in patients with detrusor-sphincter dyssynergia also revealed that anal distension for 30 s could significantly reduce the spasticity of the EUS without affecting the detrusor pressure (20). Not only the reflex activity of the urethra itself, in this study, we also demonstrated that anal distension attenuated and eventually abolished the pelvic-urethra reflex potentiation, a novel form of activity-dependent reflex potentiation, in a dose-dependent manner. This result implying anal stretch may also modulate the activity-dependent physiological/pathological response of the urethra via cross-organ innervation at the lumbosacral spinal cord levels.

Incomplete bladder emptying in patients with detrusor-sphincter dyssynergia is often related to an increase in the EUS activity during detrusor contraction. Such a dyssynergic sphincter contraction increases outlet resistance, which in turn contributes to an increased intravesical pressure and residual urine (64). In the present study, cystometric investigation demonstrated that anal distension may abolish the EMG activity of the urethra induced by bladder distension. These data offer neurophysiological evidence that anal distension can be an effective way to relax EUS when the urinary bladder was filled and may be used as an adjunct to assist voiding in patients with detrusor-sphincter dyssynergia. In addition, GABAergic neurotransmission seem to be a possible adjuvant therapeutic target for the treatment of voiding dysfunction caused by dyssynergic or overactive sphincter.

In summary, the results in this study demonstrated that anal stretch might abolish the repetitive stimulation-induced potentiation in the pelvic-urethra reflex, which is presumed to be essential for establishing urethra resistance. These data offer neurophysiological evidence that anal stretch can be an effective way to relax overactive EUS. In addition, GABAergic inhibitory neurotransmission is important in the neural mechanisms underlying EUS activity inhibited by anal stretch.

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