Spinal glutamatergic NMDA-dependent pelvic nerve-to-external urethra sphincter reflex potentiation caused by a mechanical stimulation in anesthetized rats

Jui-Miaw Liao,1,2,6 Pei-Chen Huang,3 Shwu-Fan Pan,4 Mei-Jung Chen,5 Kwong-Chung Tung,6 Hsien-Yu Peng,1,6 Jyh-Cherng Shyu,1 Ying-Ming Liou,2 Gin-Den Chen,2,6 and Tzer-Bin Lin1,8

1 Department of Physiology, College of Medicine and 2Department of Obstetrics and Gynecology, Chung-Shan Medical University Hospital, Chung-Shan Medical University, Taichung; 3Department of Life Science, National Chung-Hsing University, Taichung; 4School of Medicine, Kao-Hsiung Medical University, Kaohsiung; Departments of 4Biotechnology and 5Biomedical Engineering, Ming-Chuan University, Taoyuan; 6Department of Veterinary Medicine, College of Veterinary Medicine, Chung-Hsing University, Taichung, Taiwan; and 8Department of Medical Education, Saint Paul’s Hospital, Taoyuan, Taiwan

Submitted 7 November 2006; accepted in final form 5 January 2007

Liao J-M, Huang P-C, Pan S-F, Chen M-J, Tung K-C, Peng H-Y, Shyu J-C, Liou Y-M, Chen G-D, Lin T-B. Spinal glutamatergic NMDA-dependent pelvic nerve-to-external urethra sphincter reflex potentiation caused by a mechanical stimulation in anesthetized rats. Am J Physiol Renal Physiol 292: F1791–F1801, 2007. First published February 6, 2007; doi:10.1152/ajprenal.00443.2006.—The current study investigates whether the spinal pelvic nerve-to-external urethra sphincter (EUS) reflex potentiation can be induced by a mechanical stimulation and whether the glutamatergic mechanism is involved in yielding such a reflex potentiation. The external urethra sphincter electromyogram (EUSE) activity, evoked by a single or by repetitive pelvic nerve stimulation, in 30 anesthetized rats was recorded with/without bladder saline distension. Without saline distension (0 cmH2O), a single pulse nerve stimulation evoked a single action potential in the reflex activity, whereas repetitive pelvic stimulation and saline distension (6–20 cmH2O) both elicited a long-lasting reflex potentiation (20.05 ± 3.21 and 75.01 ± 9.87 spikes/stimulation, respectively). The saline distension-induced pelvic nerve-to-EUS reflex potentiation was abolished by 3,5-diaminopyridine (3,5-DAP) both induced a long-lasting pelvic nerve-to-EUS reflex potentiation at 20.05 ± 3.21 and 75.01 ± 9.87 spikes/stimulation, respectively. The saline distension-induced pelvic nerve-to-EUS reflex potentiation was abolished by 3,5-diaminopyridine (3,5-DAP) both induced a long-lasting pelvic nerve-to-EUS reflex potentiation at 20.05 ± 3.21 and 75.01 ± 9.87 spikes/stimulation, respectively. The saline distension-induced pelvic nerve-to-EUS reflex potentiation was abolished by 3,5-diaminopyridine (3,5-DAP) both induced a long-lasting pelvic nerve-to-EUS reflex potentiation at 20.05 ± 3.21 and 75.01 ± 9.87 spikes/stimulation, respectively. The saline distension-induced pelvic nerve-to-EUS reflex potentiation was abolished by 3,5-diaminopyridine (3,5-DAP) both induced a long-lasting pelvic nerve-to-EUS reflex potentiation at 20.05 ± 3.21 and 75.01 ± 9.87 spikes/stimulation, respectively.
tion function of lower urinary tract including the urinary bladder (52, 59–61, 65) and urethra (10, 11, 59, 62) have revealed a important role of glutamate in neural control of voiding function at the spinal cord level. In our previous studies, intrathecal application of N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate (AMPA) receptor antagonists could block the SRP. These results imply that such a stimulation-elicted SRP may share a similar neural mechanism with the widely known long-term potentiation (39, 40). Therefore, in this study, we also try to elucidate on whether SRP that is caused by a natural type of stimulation is associated with glutamatergic neural transmission.

We hypothesize that SRP may also be elicited by a mechanical stimulation and this reflex potentiation may work through the glutamatergic mechanism. The purposes of the current study are 1) to clarify whether SRP can be induced by saline distension in the urinary bladder and 2) to determine whether the glutamatergic mechanism may also be involved in the saline distension-induced SRP.

MATERIALS AND METHODS

Animal preparations. Thirty adult female Wistar rats weighing 180–350 g were anesthetized with urethane (1.2 g/kg ip). Urethane was chosen because it lacks ganglionic blocking properties and allows neural inputs to/from the viscera to be maintained. The animal care and experimental protocols were approved by the National Science Council in Taiwan. The trachea was intubated to keep the airway patent. A PE-50 catheter (Portex, Hythe, Kent, UK) was placed in the left femoral vein for administration of anesthetics when needed. Body temperature of 37°C. To prevent the escape of fluid through the apex of the bladder dome and was secured with cotton thread. In some experiments, two 4–0 nylon sutures were placed around the bladder trigone and ligated for the recording of the intravesical pressure (IUP). A wide-bore urethra cannula was inserted through the opening of the urethra and then connected to another pressure transducer and continuously recorded on the computer system.

Nerve dissection. The right pelvic nerve was carefully dissected from the surrounding tissues and was transected distally. The left pelvic nerve was also dissected and was split into several bundles for electrical stimulations. Electric shocks were used to test these nerve bundles. In case of a stable reflex activity being induced by one of the bundles, the other bundles were transected as proximally as possible. The stimulated nerve and the electrodes were bathed in a pool of warm paraffin oil (37°C) to prevent drying.

Intravesical pressure and intravesical pressure recording. A midline abdominal incision was made to expose the pelvic viscera. Both ureters were ligated distally and cut proximally to the sites of ligation. The proximal ends of the ureters drained freely within the abdominal cavity. A wide-bore cannula, with a sidearm for pressure measurement, was inserted into the lumen of the urinary bladder at the apex of the bladder dome and was secured with cotton thread. In repetitive stimulation experiments, the open end of the catheter was transected to ensure whether the reflex activity was induced by pelvic afferent fibers.

Application of drugs. Drugs were administered by intrathecal injection with a solution of known drug concentrations as described previously (47). Drugs used were APV (a glutamatergic NMDA receptor antagonist, 100 μM, 10 μM, Sigma), 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (NBQX; a glutamatergic AMPA receptor antagonist, 100 μM) and H9251 (a glutamatergic AMPA receptor antagonist, 100 μM) to determine whether SRP can be induced by saline distension in the urinary bladder and then withdrawn, leaving the electromyogram wire embedded in the muscle. The external urethral sphincter electromyogram (EUSE) activities were amplified 20,000-fold and filtered (high-frequency cut-off at 3,000 Hz and low at 30 Hz, respectively) by a preamplifier (Grass P511AC, Cleveland, OH) and then continuously displayed on an oscilloscope (Tectronics TDS 3014, Wilsonville, OR) and a recording system with a sampling rate of 20,000 Hz (MP30, Biopac).

Experimental protocols. The schematic arrangement of IVP, IUP, and EUSE recordings in response to an intact (left) or an afferent (right, the central stump) pelvic nerve fiber stimulation is shown in Fig. 1A. The volume reservoir (VR) connected to the bladder cannula was adjusted to be at the identical height as the midline of the urinary bladder at the beginning of the experiments. Once the electrodes’ positions were optimized, recording of EUSE activities began. An electric current of square wave pulses with pulse durations of 0.1 ms was applied from a stimulator (Grass S88) through a stimulus isolation unit (Grass SIU5B) and a constant current unit (Grass CCCU1A). Single shocks at fixed suprathreshold strengths (5–30 V) were repeated at 30-s intervals (referred to as the test stimulation) and given through a pair of stimulation electrodes before saline distension. This frequency of stimulation was chosen for sampling data because it did not result in response facilitation. The intensity of stimulation was gradually increased from 0 to 30 V and a stimulus intensity that yielded a single spike action potential in the EUSE was usually chosen to standardize the baseline reflex activity for 30 min. After a resting period (usually 30 min), repetitive stimulation (1 Hz, lasting for 30 min) with the intensity identical to the test stimulation was used to induce SRP. After another 30-min resting period, tests of saline distraction in the urinary bladder on reflex activities were carried out by raising the height of the reservoir in a 2-cm sequence and maintained for 1–2 min at each level until reaching 20 cm higher than the baseline position. If there were bursts of discharges in the EUSE, which indicates a voiding reflex occurred, the reservoir was not elevated any more and the test range was below this level. After all the experimental protocol had been completed, the stimulated bundle of pelvic nerves was transected as distally as possible. The central stump of this bundle was then stimulated with the test stimulation once again to ensure whether the reflex activity was induced by pelvic afferent fibers.
receptor antagonist, 20 μM, 10 μl, Sigma), bicuculline (a GABAergic antagonist), glutamate (0.1 mM, 10 μl, Sigma), and NMDA (0.1 mM, 10 μl, Sigma). Artificial cerebrospinal fluid of identical volume to tested agents was dispensed intrathecally to serve as the vehicle.

Data analysis. All data in the text and figures are means ± SE. Statistical analysis of the data was performed by means of ANOVA followed by a Student t-test. In all cases, a difference of \( P < 0.05 \) was considered as a statistically significant difference.

RESULTS

Complete data were obtained from 30 rats for statistical analysis. The test stimulation, the repetitive stimulation, and IVP manipulations were tested on all the animals. There were six animals excluded from analysis, including one rat that did not reach complete protocol and five rats where the tip of the intrathecal cannula deviated by more than 0.5 mm from the target structure.

Baseline reflex activities. The baseline reflex activity, evoked by a single test stimulation (1/30 Hz, indicated by a triangle at the bottom) on an intact pelvic nerve without saline distension (i.e., at 0 cmH_2O of IVP), was obtained from one of 24 rats and is shown in Fig. 1B, top. A single test stimulation on the intact pelvic nerve induced an action potential at a relatively constant latency to the stimulation. The mean reflex latency of the reflex activities evoked by an intact nerve stimulation was 53.13 ± 6.26 ms (Fig. 1C, \( n = 24 \)). In addition, the spike numbers in the reflex activities elicited by the test stimulation on the intact pelvic nerve showed no statistical difference over the testing period (Fig. 1D). After all the experimental procedures had been completed, the stimulated nerve was carefully transected and the central stump of this nerve fiber was stimulated with the test stimulation once again to ensure that such a reflex activity was induced by the pelvic afferent fiber. As shown in the lower trace of Fig. 1B, a single action potential with similar amplitude and shape as that evoked by an intact pelvic nerve fiber (intact) was elicited by the afferent (afferent) pelvic nerve stimulation. The latency of the reflex activity evoked by the test stimulation on the afferent pelvic nerve (afferent) showed no statistical difference with that of the intact nerve (Fig. 1C, 52.35 ± 5.58 ms). In addition, the spike number evoked by the afferent (afferent) and intact (intact) pelvic nerve fibers showed no statistical difference over the stimulation period (Fig. 1D).

Repetitive stimulation-induced reflex potentiation. As shown in Fig. 1B, the baseline reflex activity was evoked by electrical

---

**Fig. 1.** Baseline pelvic nerve-to-external urethra sphincter reflex activities. A: schematic arrangement of intravesical pressure (IVP), intraurethral pressure (IUP), and external urethral sphincter electromyogram (EUSE) recording in response to an intact (left) or an afferent (right, cut central stump) pelvic nerve stimulation (Stim) with/without saline distension in the urinary bladder connected to a volume reservoir (indicated by an arrow). B: single pulse of pelvic nerve stimulation (marked by a triangle) on an intact (top) and an afferent (bottom) pelvic nerve evoked a single action potential without saline distension (0 cmH_2O IVP). C: bars represent the latencies of the reflex activities induced by the intact and the afferent pelvic nerve stimulations (\( P > 0.05 \), between the intact and the afferent pelvic nerve stimulations). D: spike number of EUSE counted within 15 s induced by intact (○) and afferent (●) pelvic nerve stimulations (\( P > 0.05, \ n = 24 \)).
shocks of single pulses derived at a frequency of 1/30 Hz (the test stimulation) without saline distension (i.e., at 0 cmH2O of IVP). The baseline reflex activities caused by the test stimulation varied little over a 30-min testing period (Fig. 1D, circle). However, as shown in Fig. 2, A and B, a longer-lasting reflex potentiation was induced by the repetitive nerve stimulation (1 Hz, indicated by triangles at the bottom) at the same intensity as the test stimulation without saline distension. In Fig. 2C, the evoked action potentials increased gradually following the repetitive stimulation onset, then reached a plateau at ~10 min, and then were maintained at this level until the cessation of stimulation. Mean spike numbers counted within each second and induced by the repetitive intact nerve stimulation increased significantly (20.05 ± 3.21 spikes/stimulation at 30 min after stimulation onset, P < 0.01, n = 24) compared with the baseline activities induced by the single test stimulation (Fig. 2C).

Saline distension-induced reflex potentiation. The reflex activities evoked by the test stimulation (1/30 Hz, indicated by triangles at the bottom) on an intact nerve with incremental IVP (from 0 to 20 cmH2O) caused by bladder saline distensions are shown in Fig. 3. In association with the IVP elevation, the reflex activity, evoked by the test stimulation, increased and lasted longer (Fig. 3, A and B). This reflex activity ceased when we briefly turned off the stimulator and was activated once again if we turned on the switch again (data not shown), indicating that such a reflex activity was driven by the electric stimulation. In most of the rats we tested (22/24, 91.6%), the duration of the evoked activity caused by the nerve stimulation elongated and gradually transformed from a phasic firing into a tonic firing. In these cases, the numbers of action potentials evoked by each electric shock were calculated by the spike count within 15 s after stimulation minus that obtained 15 s before stimulation. Mean spike numbers in the reflex activities counted within 15 s following the electric shock increased significantly from 6 to 16 cmH2O compared with the baseline reflex activity induced by a single test stimulation without saline distension (0 cmH2O of IVP), and then the spike numbers appeared to reach a plateau level from 16 to 20 cmH2O of IVP (Fig. 3C).

Spinal cord transections. Two different procedures for spinal cord transections were performed in six rats to rule out the possibility of descending influences from the supraspinal structures. In three rats, a spinalization after a saline distension-induced reflex potentiation had been established showed no effects on such an established potentiation. In the other three rats, a potentiation in the reflex activities was also induced by a saline distension after a spinal transection. The spike numbers in saline distention-induced reflex potentiation in an intact spinal cord and transected animals

---

Fig. 2. Pelvic nerve-to-external urethra sphincter reflex potentiation caused by the repetitive pelvic nerve stimulation. A: following the onset of the repetitive intact nerve stimulation (RS; 1 Hz, marked by the triangles), a longer-lasting reflex potentiation was gradually induced in the EUSE activities without saline distension (0 cmH2O IVP). # or ## Is the same as in B. B: evoked reflex activities marked by # and ## are shown by using a faster time base. C: mean spike numbers (means ± SE, n = 24) of the reflex activity counted within each second induced by the repetitive intact nerve stimulation. **Significantly different from the baseline reflex activity induced by a single test stimulation (P < 0.01).
showed no statistical difference (75.01 ± 9.87 vs. 70.82 ± 7.72 spikes/stimulation, \( P > 0.05 \)).

**Glutamatergic and GABAergic antagonists.** Although saline distension at low IVP levels (6, 8, 10, 12, and 14 cmH\(_2\)O) induced reflex potentiation in the EUSE, to elucidate the effects of glutamatergic and GABAergic antagonists on the saline distension-induced reflex potentiation, the pressure reservoir was maintained at 16 cmH\(_2\)O higher than the baseline IVP. This pressure level was usually able to induce a maximal potentiation in the reflex activities but did not induce background tonic discharges. The reflex activities evoked by a single test stimulation (1/30 Hz, indicated by a triangle at the bottom) on an intact nerve with saline distension (Fig. 4A DIS, at 16 cmH\(_2\)O of IVP) showed more spikes and lasted longer when compared with the results of the test stimulation without saline distension (Fig. 4A control, at 0 cmH\(_2\)O of IVP). The longer-lasting saline distension-induced potentiation lessened after administration of NBQX (DIS+NBQX) and APV (DIS+APV). Mean spike numbers in the reflex activities induced by the test stimulation with saline distension (at 16 cmH\(_2\)O of IVP) after the administration of Bicuculline (DIS+Bicuculline, 53.62 ± 15.54 spikes/stimulation, \( P > 0.05 \), \( n = 24 \)). However, there were no changes in the reflex activity induced by the test stimulation with saline distension (at 16 cmH\(_2\)O of IVP) after the administration of bicuculline (DIS+Bicuculline, 53.62 ± 15.54 spikes/stimulation, \( P > 0.05 \), \( n = 24 \)).

**Effects of glutamatergic agonists.** A single pulse of test stimulation (1/30 Hz, indicated by a triangle at the bottom) on an intact nerve without saline distension (at 0 cmH\(_2\)O of IVP) evoked a single action potential (Fig. 5A control). Intrathecal administration of glutamate (Glu; 100 \( \mu \)M, 10 \( \mu \)l) and NMDA (100 \( \mu \)M, 10 \( \mu \)l) both induced a longer-lasting reflex potentiation, which is similar to what the saline distension did. Mean spike numbers in the reflex activities induced by a single pulse nerve stimulation with saline distension and counted within 15 s (Fig. 4B DIS, 71.88 ± 16.86 spikes/stimulation, \( n = 24 \)), decreased significantly after administration of NBQX and APV (DIS+NBQX, 26.16 ± 7.27 and DIS+APV, 1.72 ± 0.31 spikes/stimulation, respectively; \( P < 0.01 \), \( n = 24 \)). However, there were no changes in the reflex activity induced by the test stimulation with saline distension (at 16 cmH\(_2\)O of IVP) after the administration of bicuculline (DIS+Bicuculline, 53.62 ± 15.54 spikes/stimulation, \( P > 0.05 \), \( n = 24 \)).
Secondary changes resulting from the reflex potentiation. As shown in Fig. 6B, a single pulse test stimulation (1/30 Hz, indicated by a triangle at the bottom) on an intact nerve with saline distension (DIS, maintained at ~12 cmH2O of IVP) evoked series of longer-lasting firing in the reflex activities, compared with that did by the identical stimulation without saline distension (Fig. 6A Control, at 0 cmH2O of IVP). Furthermore, the duration of the contraction wave in the IUP, secondary to the sphincter muscle contraction driven by the reflex firing, increased in a parallel fashion, whereas the peak pressure of the IUP remained unchanged.

DISCUSSION

Our results show that a single pulse of nerve stimulation can evoke a single action potential on the EUS and repetitive nerve stimulations can elicit a long-lasting SRP in the pelvic nerve-to-EUS reflex activities. The SRP originating from the afferent pelvic nerves cannot only be induced by repetitive electric shocks but also by a type of mechanical stimulation, i.e., saline distension in the urinary bladder. Glutamatergic antagonists including NBQX and APV can inhibit the saline distension-induced reflex potentiation. Meanwhile, glutamatergic agonists including glutamate and NMDA can also induce SRP without saline distension. We also demonstrated that the SRP caused...
by the mechanical stimulation may modify activities of the urethra, which is the target organ of the pelvic nerve-to-EUS reflex activity.

A repetitive activation of synaptic connections leading to the modulation of synaptic transmissions is found in a variety of brain structures (32, 43, 50). The long-term potentiation, which is characterized by a prolonged increase in the excitability of CA1 neurons, has been widely explored by researchers (9, 31, 47). However, any biological function of the activity-dependent reflex potentiation still seeks final proof partly because it has not been shown that the pattern and timing of a naturally occurring afferent barrage can induce robust reflex potentiation (1, 41, 49). In this study, we used a mechanical stimulation of saline distension in the urinary bladder to induce SRP in the pelvic nerve-to-EUS reflex activities instead of electric stimulations or pharmacological manipulations. Our result implies that substantial changes in the pelvic nerve-to-EUS reflex activities can be induced under a simulated physiological condition.

The SRP on the pelvic nerve-to-EUS reflex activities induced by changes in IVP in the present study cannot be simply explained by an augmented reflex activity in response to a recruitment of afferent inputs due to the IVP increment. The following findings in our study may support our working hypothesis. The evoked activities increased following IVP increments; however, most of the units we tested in this study remained inactive below 16 cmH2O. This finding indicates that such a recruitment of afferent fibers is not yet sufficient enough to induce reflex activities (i.e., spontaneous firing). In addition, we also revealed that the firing periods of the evoked activity, elongated by elevation in the

---

**Fig. 5. Glutamatergic agonist-induced pelvic nerve-to-external urethra sphincter reflex potentiation without saline distension.**

**A:** EUSE activities evoked by the test stimulation (1/30 Hz, marked by the triangles) on the intact pelvic nerve without saline distension (control, 0 cmH2O of IVP). Without saline distension, intrathecal glutamate (Glu) and NMDA induced reflex potentiation. Recording traces with a faster time base are shown on the right. **B:** mean spike numbers (means ± SE, n = 24) induced by the test stimulation on the intact pelvic nerve (control) and by the test stimulation with intrathecal administrations of glutamate or NMDA (Glu and NMDA, respectively). **Significantly different from the control group (P < 0.01).
IVP levels, were so long (may last for seconds, as shown in Fig. 3A), that it cannot be explained by just a subliminal fringe state because the excitatory postsynaptic potential decays for only milliseconds. Therefore, the possible mechanism underlying the saline distension-induced SRP in this study would be an increment in the reflex efficacy rather than simply a recruitment of afferent fibers. Furthermore, when the IVP, caused by the saline distension, is higher than 16 cmH2O (i.e., 18 and 20 cmH2O; Fig. 3A), it is sufficient to induce background firing, and the evoked activity holds a relatively constant value rather than an increase following the IVP increment (Fig. 3B).

This result indicates, despite the growing number of afferent fibers recruited by the saline distension, that the activity-dependent SRP on the pelvic nerve-to-EUS reflex activities is not affected by the increase in recruited fibers. Considering all the above findings, we can assume that an activity-dependent reflex within the spinal cord, secondary to the IVP changes underlying the SRP, cannot be simplified as just an augmented reflex response primary to an incremental IVP. However, to obtain the electromyogram units and clearly evaluate the activity, units with background discharges below 16 cmH2O were usually discarded to avoid contamination of the evoked activity with the background tonic discharges. For this reason, although they did respond to saline distension with low IVP levels (as shown in Fig. 3B, SRP can be induced at the IVP level of 6, 8, 10, 12, and 14 cmH2O), the units recorded in the present study seem to be high threshold units. Whether these units mediated a pathological state of higher IVP should be taken into consideration.

In the present study, pelvic nerve-to-EUS reflex potentiation was shown during both silent and tonic phase of EUS electromyogram activity, which corresponds to the storage and voiding phases of the micturition cycle, respectively. This result implies pelvic nerve-to-EUS reflex play roles in not only the storage phase but also the voiding phase of micturition cycles. These results were quite correlated with our unpublished data, which demonstrated the pelvic nerve-to-EUS reflex potentiation was induced in both producing urethra closure during the storage phase and after a voiding contraction as well as
developing sufficient pressure gradient to facilitate urine emission during the ascending phase of a voiding contraction.

Randić et al. (47) reported that the tetanization-induced enhancement of excitatory postsynaptic potentials may be related to the mechanisms involved in the generation of postinjury pain hypersensitivity. Many researchers have also reported several chemical irritation-induced hyperactivities in the urethral sphincter (14, 53). A high resistance in the lower urinary tract, as a result of a hyperactive sphincter, is considered to be a cause of obstructive bladder dysfunctions (33). In the present study, various levels of saline distension were used to induce SRP on the pelvic nerve-to-EUS reflex activities. The IVPs, we tested, were lower than 20 cmH2O, which are within the physiological range of bladder distension (15). However, the bladder is an organ with high compliance and it can be filled to large volumes under a low pressure. In the present study, we elevated the IVP level to as high as 20 cmH2O, which required a considerable volume (range from 0.6 to 1.2 ml). Although this dose does not detract from the validity that SRP on the pelvic nerve-to-EUS reflex activities can be induced at a low distension pressure (i.e., started at 6 cmH2O of IVP). The possibility that the induction of SRP may be involved in pathological conditions, such as bladder overdistension caused by benign prostate hypertrophy, should also be taken into consideration.

In addition, Lagos and Ballejo (37) investigated cyclophosphamide-induced cystitis and suggested that a reflex plasticity within the spinal cord may be one of the important pathways, which elicit a hypersensitive bladder. Therefore, it cannot be ruled out that there is the possibility that the reflex plasticity recorded in the present study is involved in other pathological conditions.

Using a urinary bladder with a transected pelvic nerve, we reported that repetitive (39) and tetanic (40) electric shocks on the central stump of the pelvic nerve produced SRP on the pelvic nerve-to-EUS reflex activities. However, since the pelvic nerve was transected, it was not easy to investigate whether physiological/pathological stimulation on the bladder wall affects SRP. In the present study, instead of using the urinary bladder with a transected afferent nerve, we set up an experimental model where the afferent pathway was left intact and, therefore, the impact of the physiological/pathological stimulations on the bladder wall on SRP could be explored. On the other hand, to maintain the afferent pathways for bladder distension to induce a SRP, an intact pelvic nerve was stimulated instead of a central stump in this study. The possibility of direct efferent impulses inducing electromyogram activities can be ruled out because of the following evidence. 1) Reflex latency recorded in this study was 53.3 ± 6.26 ms which is too long for the somatic pudendal nerve to elicit activities in the EUS. 2) The pelvic efferent fibers innervate the detrusor rather than the urethra. Activities of the urethra sphincter muscles are not affected by the pelvic efferent fibers. 3) The stimulating parameter sufficient to induce the detrusor contraction via the postganglionic parasympathetic nerve fibers is 5 Hz for 30 s or higher (35, 36). Single impulses with pulse durations of 0.1 ms used in this study are not able to yield bladder contractions. We consider that firings in the electromyogram were not caused secondarily by the detrusor contraction. 4) At the end of the experiments, the test stimulation on the central stump of the pelvic nerve evoked an action potential with identical amplitude, shape, and time latency as the intact nerve did. In addition, no statistical difference was found between the baseline reflex activities evoked by the intact and afferent pelvic nerve stimulations. Based on the above evidence, we think that the activities recorded in this experiment are mainly the result of pelvic afferent inputs.

On the other hand, although the sympathetic hypogastric nerve does not innervate the EUS, it may induce urethra closure via the activation of the internal urethra sphincter during the storage phase of a micturition cycle. Our results cannot exclude whether the pelvic nerve-to-hypogastric nerve reflex participates in the distension-induced elongation in the IUP wave because we did not record blood pressure or hypogastric nerve activity during our experiments. As shown in Fig. 6, small contraction waves in the IUP had a close relationship with the spike activity in the EUS, implying that these contraction waves were mainly the result of the contraction of the somatic EUS, which is innervated by the pudendal nerve, although the effects of the sympathetic nerve cannot be excluded.

Glutamate is a widely utilized neurotransmitter in the lumbosacral spinal cord, at which mediating the function of the lower urinary tract including urinary bladder and urethra. In vivo animal investigations revealed that the activity of the external urethra sphincter is sensitive to glutamatergic NMDA and AMPA neurotransmission (21, 22). Pharmacological studies suggested glutamate is not only essential for the physiological function of the lower urinary tract (52, 59, 60, 62, 63, 65) but is also a possible therapeutic target in urinary dysfunctions resulted from spinal cord injury (64) and cerebral infraction (58). These findings are quite correlated with the results in the present study that glutamate may participate in the reflex activity of the urethra. In addition, the activity-dependent SRP can be also affected by the glutamatergic antagonist indicated a potential role of glutamatergic agonist/antagonist in further investigations on the therapeutic agents for urinary tract dysfunctions such as the Hinman syndrome (26), in which the external urethra sphincter failed to relax during voiding in the absence of overt neurological lesion.

The induction of long-term potentiation is presently thought to activate glutamatergic NMDA and AMPA receptors (2, 16, 19, 24, 34, 44). In this study, NMDA and AMPA receptor antagonists attenuated SRP suggesting that distension-induced SRP may share a similar mechanism with long-term potentiation (23). This conjecture is in accordance with a recent report showing that the strength of the glutamatergic primary afferent transmission of the spinal dorsal horn neurons might be potent following tetanic peripheral inputs (47). However, the multiple fibers recording technique used in this study is a limitation, so it is difficult to conclude whether this enhancement is mediated by a “long-term potentiation-like” synaptic transmission. Further investigation of the synaptic efficacy on the dorsal horn neurons within the spinal cord is needed.

Although there is little to suggest that inhibitory mechanisms contribute to the maintenance of synaptic long-term potentiation (25), there is evidence that its induction is affected by inhibitory influences (20, 54). In this study, the SRP induced by the bladder saline distension was not affected by the intrathecal application of bicuculline, a GABAA receptor antagonist. We can rule out the involvement of the GABAA polysynaptic inhibitory pathway. However, the role of other...
GLUTAMATE-MEDIATED REFLEX POTENTIATION

inhibitory neuromodulations, such as GABA<sub>B</sub>, serotonin, and glycine, in the SRP still needs further investigation.

GRANTS
This research was supported by the National Science Council in Taiwan (NSC 94-2320-B-040-026, NSC 94-2320-B-040-026) and Chung-Shan Medical University (93-OM-B-038, 94-OM-B-032).

REFERENCES

63. Yoshiyama M, Erickson KA, Erdman SL, de Groat WC. Effects of N-methyl-D-aspartate (dizocilpine) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (LY215490) receptor antagonists on the voiding reflex induced by perineal stimulation in the neonatal rat. *Neuroscience* 90: 1415–1420, 1999.