Spinal glutamatergic NMDA-dependent cyclic pelvic nerve-to-external urethra sphincter reflex potentiation in anesthetized rats

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Liao J-M, Yang C-H, Cheng C-L, Pan S-F, Chen M-J, Huang P-C, Chen G-D, Tung K-C, Peng H-Y, Lin T-B. Spinal glutamatergic NMDA-dependent cyclic pelvic nerve-to-external urethra sphincter reflex potentiation in anesthetized rats. Am J Physiol Renal Physiol 293: F790–F800, 2007. First published March 20, 2007; doi:10.1152/ajprenal.00296.2006.—The purposes of this study were to investigate whether the pelvic nerve-to-external urethra sphincter (EUS) reflex potentiation can be induced under physiological conditions and to determine whether glutamatergic neurotransmission is involved in the reflex potentiation. Stimulation-evoked reflex activities, during rhythmic bladder contractions caused by a continuous saline infusion, in 21 anesthetized rats were recorded with/without the intrathecal administration of 10 μl of CNQX (a glutamatergic AMPA receptor antagonist; 100 μM) and APV (a glutamatergic NMDA receptor antagonist; 100 μM). Reflex activities became potentiated following the increment of intravesical pressure (IVP) during the storage phase (2.39 ± 0.28 spikes/mmHg, n = 21) and the ascending period of the voiding phase (1.46 ± 0.35 spikes/mmHg, n = 21) and decreased following the decrement of IVP during the descending period of the voiding phase (1.50 ± 0.33 spikes/mmHg, n = 21). Although it is characterized by a low IVP, a postvoiding reflex potentiation in stimulation-evoked activities was elicited at the critical period after a voiding contraction had just finished (23.95 ± 8.96 spikes/mmHg, n = 21). The slope of the regression line of evoked activities vs. the IVP during the storage phase was significantly (P < 0.01) higher than that of the ascending and descending periods of the voiding phase, but there was no statistical difference between the ascending and the descending periods (P > 0.05). In addition, the slope of the regression line of postvoiding reflex potentiation was significantly higher than that of the storage phase (P < 0.01). All the slopes of the regression lines decreased after intrathecal CNQX administration (from 3.15 ± 0.44, 2.10 ± 0.57, 2.13 ± 0.53, and 21.30 ± 3.41 to 0.83 ± 0.31, 0.74 ± 0.12, 0.76 ± 0.12, and 4.31 ± 3.71 spikes/mmHg in storage, ascending and descending period of the voiding phase, and postvoiding potentiation, respectively; all P < 0.01, n = 10). The slopes of the regression lines became almost horizontal after intrathecal APV administration (from 3.15 ± 0.44, 2.10 ± 0.57, 2.13 ± 0.53, and 21.30 ± 3.41 to 0.16 ± 0.12, 0.21 ± 0.07, 0.18 ± 0.05, and 0.23 ± 0.76 spikes/mmHg in storage, ascending and descending period of the voiding phase, and postvoiding potentiation, respectively; all P < 0.01, n = 10). Our results suggest that a potentiation in the pelvic nerve-to-EUS reflex can be induced under physiological conditions and the glutamatergic mechanism appears to be involved in this reflex potentiation.

postvoiding potentiation; posttetanic potentiation; glutamate

ACTIVITY-DEPENDENT REFLEX plasticity means that the efficacy of a reflex can vary depending on the patterns of ongoing activities (42). Long-term potentiation (5–7) and posttetanic potentiation (9, 44) are characterized by a long- and a short-lasting enhancement in efficacy of excitatory synapses following a strong brief stimulation of input fibers, respectively. Many studies exploring long-term potentiation have focused on the CA1 area of the hippocampus (40, 51) and have suggested that long-term potentiation is involved in the laying down of memory traces, partly because the hippocampus is the area known to be necessary for the formation of declarative memory and partly because long-term potentiation fulfills the requirements of Hebb’s model of memory (41, 47, 48). It is clear, however, that long-term potentiation does not only occur in the hippocampus but is also widespread in the central nervous system including the spinal cord (43). Studies investigating activity-dependent reflex potentiation in the spinal cord suggest that long-term potentiation in the spinal cord area might play a crucial role in hyperalgesia and allodynia (45, 52–54).

Activity-dependent reflex plasticity can be elicited in different ways, such as applying brief high frequency (10, 24), applying repetitive electric shocks to the pathways (19), and using direct pharmacological manipulations (18, 20, 26). However, few studies have tested whether an activity-dependent reflex plasticity can be induced under physiological conditions where changes are made in stimulation to a specific organ with intact functions. For example, would changes in intravesical pressure at various stages of a micturition cycle produce activity-dependent pelvic nerve-to-external urethra sphincter (EUS) reflex plasticity? Baba et al. (1) indicate that an activity-dependent reflex plasticity induced under a functional physiological condition may offer a gateway to elucidate the physiological/pathological relevancies of reflex plasticity in neuroscience.

Recently, in our laboratory, we have discovered and reported on an activity-dependent spinal reflex potentiation (SRP) in pelvic nerve-to-EUS reflex activity. By using in vivo animal

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preparations, we have demonstrated that low-frequency repetitive (14, 15, 33–35) and tetanic (36) stimulations on the afferent nerve fiber may induce SRP. The in vivo animal preparations used in our studies have an intact spinal cord with dorsal and ventral roots attached; therefore, the neural network within the central nervous system remains intact and offers us a chance to investigate the activity-dependent reflex plasticity under physiological conditions, which is quite different from investigations using brain/spinal slices.

In addition, our previous studies showed that intrathecal application of N-methyl-D-aspartic acid (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonists blocked or attenuated the SRP that was induced by repetitive stimulation. These results indicate that the SRP induced by repetitive afferent fiber stimulation may share a similar glutamatergic NMDA-dependent mechanism with the well-known long-term potentiation (14, 15, 36). Researchers have reported that glutamate is involved in the afferent limb of the spinal reflex at the lumbosacral levels (4). Pharmacological investigations have revealed that the activities of the lower urinary tract including the urinary bladder (49, 56–60, 62) and urethra (11–13, 56, 59) were inhibited by glutamatergic receptor antagonists, indicating a important role of glutamate in the lumbosacral spinal cord mediated micturition activity. Therefore, whether the SRP induced under physiological conditions involve glutamatergic mechanism is an interesting issue that needs further investigation. We hypothesized that SRP may be elicited during rhythmic bladder micturition contractions and that this SRP may take place via the glutamatergic mechanism. The purposes of this study were to clarify whether the SRP can be induced during rhythmic micturition cycles and to determine whether the glutamatergic mechanism is involved in the induction of the SRP.

MATERIALS AND METHODS

Animal preparations. Twenty-five adult female Wistar rats weighing, 280–350 g, were anesthetized with urethane (1.2 g/kg ip). Urethane was chosen because it lacks gangliionic blocking properties and allows the maintenance of neural inputs to/from the viscera. 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 was kept at 36.5–37.0°C by infrared light and was monitored using a rectal thermometer. The rats were monitored for the corneal reflex and a response to noxious stimulation to the paw throughout the experiment. If responses were present, a supplementary dose (0.4 g/kg iv) of urethane was given through the venous catheter. When the experiments were completed, the animals were killed by the numbers of action potentials, in the EUS electromyogram, evoked by pelvic nerve stimulation at one-fourth, one-half, and the full value of the threshold IVP (within the range of ±10%) during storage as well as the full value of the maximal IVP (within the range of ±10%) during voiding phases of the rhythmic micturition cycles, respectively.

External urethra sphincter electromyogram recording. Epoxy-coated copper wire (50 μm, Giken, Tokyo, Japan) electromyogram electrodes were placed in the EUS. The placement of the electrodes was performed using a 30-gauge needle with a hooked electromyogram electrode positioned at the tip (1.0–1.5 mm). The needle was inserted into the sphincter ~1–2 mm lateral to the urethra 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), then continuously displayed on an oscilloscope (Tectronics TDS 3014, Wilsonville, OR) and the recording system with a sampling rate of 20,000 Hz (MP30, Biopac, Santa Barbara, CA).

Pelvic nerve stimulation. The left pelvic nerve was dissected carefully from the surrounding tissue and was split into several bundles for stimulations without transaction. The right pelvic nerve was left intact. The schematic arrangement of IVP and EUSE recordings in response to intact pelvic nerve fiber stimulation is shown in Fig. 1A (left). 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 CCU1A). The stimulated nerve and the electrodes were bathed in a pool of warm paraffin oil (37°C) to prevent drying. 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 infusion. 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. After an equilibrium period (usually 30 min), repetitive stimulation (1 Hz, lasting for 30 min) with the intensity identical to the test stimulation was applied to induce facilitation in reflex activities. After a 30-min rest, tests of saline
infusion into the urinary bladder on reflex activities were executed in association with the application of the test stimulation to the pelvic nerve under rhythmic bladder contractions for 30–60 min, and data were then analyzed off-line. We checked the IVP values at one-fourth, one-half, and at the full value of the threshold/peak IVP (within the range 0–100 cmH2O IVP). C: bars represent the latencies of the reflex activities induced by the intact (left) and the afferent (right) pelvic nerve stimulations (P > 0.05 between intact and afferent pelvic nerve stimulations, n = 21). D: spike numbers of the EUSE counted within 15 s and induced by intact pelvic nerve test stimulations (1/30 Hz, for 30 min, P > 0.05, n = 21).

Fig. 1. Baseline pelvic nerve-to-external urethra sphincter (EUS) reflex activity induced by a test stimulation. A: schematic arrangement of intravesical pressure (IVP) and external urethra sphincter electromyogram (EUSE) recordings in response to an intact (left) or an afferent (right) pelvic nerve stimulation (Stim). B: single-pulse stimulation (arrow) on an intact (top trace) and an afferent (bottom trace) nerve fiber evoked a single EUSE action potential without saline distension (0 cmH2O IVP). C: bars represent the latencies of the reflex activities induced by the intact (left) and the afferent (right) pelvic nerve stimulations (P > 0.05 between intact and afferent pelvic nerve stimulations, n = 21). D: spike numbers of the EUSE counted within 15 s and induced by intact pelvic nerve test stimulations (1/30 Hz, for 30 min, P > 0.05, n = 21).

Application of drugs. In 12 of the 21 rats, on which pharmacological tests were carried out, drugs were administered by intrathecal injection with a solution of known drug concentrations (43). Drugs used were 6-cyano-7-nitroquinoxalin-2,3-dione [CNQX; a glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist; 100 μM, 10 μl, Sigma] and D-2-amino-5-phosphonovalerate [APV, a glutamatergic N-methyl-D-aspartic acid (NMDA) receptor antagonist; 100 μM, 10 μl, Sigma]. Artificial cerebrospinal fluid [(in mM) 118 NaCl, 3 KCl, 25 NaHCO3, 1.2 NaH2PO4, 1 MgCl2, 1.5 CaCl2, and 10 glucose, pH = 7.4] of identical volume to tested agents was dispensed intrathecally to serve as a vehicle.

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

RESULTS

Baseline reflex activities. Reflex activities elicited by the test stimulation (1/30 Hz for 30 min) on the intact pelvic nerves without saline infusion obtained from 1 of 21 rats and are shown in Fig. 1B (intact). The mean reflex time for the pelvic nerve stimulations to evoke an action potential in the EUSE was 10.220.33.6 on April 3, 2017 http://ajprenal.physiology.org/ Downloaded from
was 55.84 ± 2.57 ms (Fig. 1C, n = 21). The mean spike number elicited by the test stimulation on the intact pelvic nerves without saline infusion is summarized in Fig. 1D. No statistical difference was found in the spike numbers evoked by the nerve stimulation over the stimulation period (0.98 ± 0.21 vs. 1.02 ± 0.11 spikes/stimulation at the beginning and the end of the stimulation, respectively, P > 0.05, n = 21). After all the experiments were completed, the effects of the pelvic efferent nerve were ruled out by the following procedures: We applied a local anesthetic (2% lidocaine) topically and distal to the site of stimulation. Five minutes later, the pelvic nerve was transected and the central stump was stimulated with the identical test stimulation once again (Fig. 1A afferent). In all of the 21 rats we tested, the pelvic afferent fiber stimulation elicited an action potential with a similar amplitude and shape (Fig. 1B, afferent) to that of the intact nerve stimulation. In addition, the latency of the reflex activity induced by the afferent nerve stimulations showed no statistical difference compared with that of the intact nerve stimulations (59.34 ± 0.98 ms, P > 0.05, n = 21, Fig. 1C).

Repetitive stimulation-induced reflex potentiation. The baseline excitability of the reflex was assayed by recording the action potentials in the EUSE activities resulting from the intact pelvic nerve stimulations with electrical shocks of single pulses derived at a frequency of 1/30 Hz (test stimulation) without saline infusion. The evoked activities varied little over 30 min of testing (Fig. 1D). However, without saline infusion, a longer-lasting reflex potentiation in the pelvic nerve-to-EUS reflex activity was induced by the repetitive pelvic nerve stimulation (1 Hz, indicated by the arrows, bottom) at the same intensity as the test stimulation (Fig. 2A). As shown in Fig. 2B, the evoked activity gradually increased following the onset of repetitive stimulation and then reached a plateau at ~1 min which was then maintained until the cessation of stimulation. The reflex activities were enhanced by repetitive stimulation in 21 of 25 rats. These animals were used for further study and statistical analysis. Mean spike numbers, induced by the repetitive stimulation and counted within each second, increased significantly (18.32 ± 0.87 spikes/stimulation at 30 min after stimulation onset, P < 0.01, n = 21) compared with the baseline activities induced by the test stimulation (1.13 ± 0.24 spikes/stimulation at 30 min after stimulation onset, Fig. 2B).

Cyclic reflex potentiation. As shown in Fig. 3A, a continuous saline infusion (0.1 ml/min) produced rhythmic bladder contractions with bursting discharges in the EUSE during voiding. The spike numbers in the EUSE, evoked by pelvic nerve stimulation within 15 s after stimulation, were recorded to assay the reflex excitability during rhythmic bladder contractions. If background activities occurred or the activities elongated and transformed gradually from phasic firing into tonic firing, the numbers of action potentials evoked by each electric shock were calculated by counting the number of spikes 15 s after stimulation minus the number obtained 15 s before stimulation. The reflex activities evoked by the test stimulation (1/30 Hz, indicated by the arrows at the bottom of the tracing) on an intact pelvic nerve at various stages of micturition cycles are shown in Fig. 3B. During micturition cycles, the evoked reflex activities caused by the test stimulation increased and lasted longer following an increase in IVP during storage phase (Fig. 3, B and C). The mean spike numbers evoked by the test stimulation at one-fourth, one-half, and full value of the threshold IVP during the storage phase were 3.58 ± 0.62, 6.75 ± 4.03, and 10.25 ± 5.84 spikes/stimulation, respectively (n = 21). Similar to the storage period, the evoked activity increased following the increment of IVP during the ascending period of the voiding phase (Fig. 4A). The mean spike numbers evoked by the test stimulation at one-fourth, one-half, and full value of the maximal IVP during the ascending period of the voiding phase were 12.25 ± 2.07, 21.95 ± 3.71, and 34.00 ± 5.72 spikes/stimulation, respectively (n = 21). Furthermore, the evoked activities decreased and became shorter in association with a decrease in the IVP during the descending period of the voiding phase (Fig. 4B). The mean spike numbers evoked by the test stimulation at the full value, one-half, and one-fourth of the maximal IVP during the descending period of the voiding phase were 35.70 ± 5.84, 23.30 ± 3.31, and 13.55 ± 1.89 spikes/stimulation, respectively (n = 21). In addition, as shown in Fig. 3D, during the plateau period of the voiding phase, which is characterized by bursts of EUSE discharges and
high-frequency oscillations in IVP in association with urine emission, pelvic nerve stimulation did not affect the bursts of discharges during this period; i.e., the EUSE fired with a stable frequency and amplitude without any alteration elicited by pelvic nerve stimulation. The regression lines of the spike numbers evoked by test stimulations at various IVP levels during storage (i.e., 1/4, 1/2, and the full value of threshold IVP during the storage period) and voiding (i.e., 1/4, 1/2, and the full value of maximum IVP during both the ascending and descending phases) are summarized in Fig. 5D. In addition, since the test stimulation did not affect the bursts of discharges during the plateau period, the evoked activities during this period were therefore excluded from statistical analysis. As shown in Fig. 5D, the slope of the regression line within the storage period (STOR; 2.39 ± 0.28 spikes/mmHg, n = 21) was higher than the slope within the ascending phase of the voiding period (VOID-asc; 1.46 ± 0.35 spikes/mmHg, n = 21, P < 0.05), while no statistical difference was found between slopes of regression lines within ascending and descending periods (VOID-des; 1.50 ± 0.33 spikes/mmHg, n = 21, P > 0.05) of the voiding phase.

Postvoiding reflex potentiation. As shown in Fig. 5A, the reflex activities evoked by pelvic nerve stimulation were enhanced following an increase in IVP during rhythmic micturition contractions. Although the IVP was low, there was an increase in the spike number evoked by the electric shock at the critical time point when a voiding contraction had just finished (Fig. 5B, mean 21.45 ± 3.36 spikes/mmHg, n = 21, P < 0.01) compared with the firing evoked at one-fourth of the threshold IVP during the storage period but with a longer latency after a voiding contraction had finished (Fig. 5C, mean 3.85 ± 0.62 spikes/stimulation, n = 21), despite that the latter was characterized by a roughly equal (or even a higher) IVP. As shown in Fig. 5D, the slope of the regression line of postvoiding potent-
tiation, i.e., reflex activity potentiation that occurred at the critical time point when a voiding contraction had just finished (23.95 ± 8.96 spikes/mmHg, n = 21), was far higher than that of the storage and voiding phases (both P < 0.01, n = 21).

Effects of glutamatergic antagonists. To elucidate the effects of glutamatergic antagonists on the cyclic reflex potentiation and postvoiding reflex potentiation, glutamatergic AMPA and NMDA antagonists, i.e., CNQX and APV, were tested via intrathecal injections. As shown in Fig. 6, both CNQX and APV elongated the storage phase and produced several insufficient contractions with a gradual increase in IVP. Eventually, a voiding contraction (marked by * in Fig. 6) characterized by urine emission was induced. The reflex activities evoked by pelvic nerve stimulation in all the periods we investigated, including the storage phase (from 10.40 ± 2.12 to 3.90 ± 1.27 spikes/stimulation at the threshold pressure, n = 10), the ascending period (from 40.90 ± 10.13 to 9.40 ± 2.27 spikes/stimulation at the maximal pressure, n = 10), and the descending period (from 42.40 ± 9.97 to 10.00 ± 2.82 spikes/stimulation at the maximal pressure, n = 10) and postvoiding reflex potentiation (from 21.30 ± 3.41 to 5.70 ± 1.59 spikes/stimulation, n = 10), decreased after CNQX was introduced and were almost abolished after the injection of APV (the storage phase, 1.70 ± 0.33 spikes/stimulation at the threshold pressure; the ascending period of the voiding phase, 3.30 ± 1.11 spikes/stimulation at the maximal pressure; the descending period, 2.10 ± 0.52 spikes/stimulation at the maximal pressure; and the postvoiding potentiation, 2.80 ± 0.91 spikes/stimulation, n = 10) compared with the data at the identical IVP before the injections (Fig. 5A, P < 0.01, n = 10). As shown in Fig. 7, the slopes of the regression lines during the storage phase (Fig. 7A, from 3.15 ± 0.44 to 0.83 ± 0.31 spikes/mmHg, P < 0.05, n = 10), the ascending period (Fig. 7B, from 2.10 ± 0.57 to 0.74 ± 0.12 spikes/mmHg, P < 0.05, n = 10), and the descending period (Fig. 7C, from 2.13 ± 0.53 to 0.76 ± 0.12 spikes/mmHg, P < 0.05, n = 10) as well as postvoiding reflex potentiation (Fig. 7A, from 16.22 ± 0.38 to 4.31 ± 3.71 spikes/mmHg, P < 0.05, n = 10) decreased significantly after the intrathecal CNQX injection. Furthermore, the slopes of the regression lines decreased and became almost horizontal after APV administration (the storage phase, 0.16 ± 0.12; the ascending period of voiding phase, 0.21 ±
0.07, and the descending period of the voiding phase, 0.18 ± 0.05; postvoiding reflex potentiation, 0.23 ± 0.76 spikes/mmHg; respectively; all P < 0.05, n = 10).

DISCUSSION

Our findings are summarized as follows: 1) Single-pulse pelvic nerve stimulation evoked an action potential in the EUSE, whereas repetitive pelvic nerve stimulations resulted in a long-lasting SRP when the urinary bladder was empty. 2) Not only repetitive electric shocks but also an increment in IVP during rhythmic micturition cycles may induce a SRP. 3) Nerve stimulation had no effect on the bursts of discharges in the EUSE during the plateau period of the voiding phase, despite the high IVP during this period. 4) Although it is characterized by a low IVP, a postvoiding reflex potentiation was elicited at the critical time point when a voiding contraction had just finished. 5) The glutamatergic AMPA antagonist attenuated and the NMDA antagonist abolished cyclic SRP and postvoiding reflex potentiation during rhythmic micturition cycles.

We think that the cyclic SRP induced by changes in IVP in this study cannot be simply explained by an augmented reflex activity in response to a recruitment of afferent inputs due to the IVP increment. This assumption was supported by the following lines of evidence. 1) Although the evoked activities increased following IVP increments during the storage phase, most of the units we tested in this study remained inactive during the early stages of the storage period (i.e., 1/4 of the threshold pressure), indicating that the recruitment of afferent inputs is not yet sufficient enough to induce reflex activities (i.e., spontaneous firing). 2) The firing periods of the evoked activity, which were elongated by an elevation in the IVP...
levels during the storage phase, were so long (as shown in Fig. 3B, they may last for seconds) that they cannot be explained by a subliminal fringe state because the excitatory postsynaptic potential decays for only milliseconds; therefore, the possible mechanism underlying the cyclic reflex potentiation in this study would be an increment in reflex efficacy rather than a simple recruitment of afferent fibers. 3) The slope of the regression line was higher during the storage phase than during the ascending period of the voiding phase. Since the slopes of the regression lines were calculated by dividing the firing frequency by the IVP (i.e., spike numbers/IVP), no statistical difference should be found between the storage and voiding phase if the potentiated reflex activities were simply results of augmented peripheral inputs. However, in the present study, the slope of the regression line during the storage phase was higher than that of the ascending period in the voiding phase, indicating the mechanisms of the reflex potentiation were not identical. 4) Although it is characterized by a minimal IVP at the time point just after a voiding contraction had just finished, the reflex activities evoked by nerve stimulation were potentiated and elongated compared with the reflex activities at one-fourth of the threshold pressure, which had about the same or even a higher IVP than the former, indicating that such a potentiated reflex activity is a result of an activity-dependent modification in reflex efficacy secondary to an IVP change but is not caused by the IVP change itself. 5) Despite the IVP being relatively higher than at any other stage, nerve stimulations failed to induce evoked activities once the bursts of discharges in the EUSE occurred during the plateau period of the voiding phase; i.e., only the spontaneous bursts of discharges without any effects caused by the electric shock were recorded. This result indicates that the reflex potentiation caused by the IVP increment failed to overcome the burst discharges integrated within spinal cord during the urine emission period of the voiding phase. Therefore, the underlying mechanism of reflex plasticity, in the present study, is not the...
IVP increment itself, but the activity-dependent changes in reflex efficacy, which can be masked by another neuronal activity. All the above evidence implies that not only is a simple augmented reflex response primary to an incremental IVP but an activity-dependent neural plasticity within the spinal cord (or higher centers) secondary to cyclic IVP changes during micturition cycles is the basis for SRP as demonstrated here.

On the other hand, the postvoiding reflex potentiation in reflex activities in this study should not be seen as a result of the posttetanic potentiation in the external urethral skeletal muscle fibers. The posttetanic potentiation in a skeletal muscle fiber is caused by insufficient calcium ion reabsorption within the muscle cell after high-frequency stimulation, so the potentiation is a result of changes in intracellular conditions. On the contrary, we recorded the postvoiding reflex potentiation in the sphincter muscle electromyogram, which means that the neural-driven electrical activities took place before the excitation-contraction coupling procedures within the muscle cell. Our finding implies that such a phenomenon was induced by a potentiated neural drive within the spinal cord or from higher neural centers instead of within the urethral muscle itself.

A repetitive activation of synaptic connections leads to modulation of synaptic transmissions in a variety of brain structures (26, 39, 47). Forms of activity-dependent reflex potentiation, including long-term potentiation and posttetanic potentiation, have been widely explored by researchers (10, 38, 43, 53, 54). Nevertheless, any biological function of activity-dependent reflex potentiation is still seeking final proof partly because it has not been shown that pattern and timing of a naturally occurring afferent barrage can induce robust reflex plasticity (2, 37, 46). Instead of electric stimulations or pharmacological manipulations, we used an in vivo preparation with intact physiological function, i.e., a urinary bladder that periodically voids, to induce SRP in the pelvic nerve-to-EUS reflex activities. Our finding leads us to believe that substantial changes in the pelvic nerve-to-EUS reflex activities can be induced under functional physiological conditions. The pelvic nerve-to-EUS reflex is essential for urine continence during micturition cycles (22, 23). Our results suggest that during the storage and the ascending period of the voiding phase, bladder distension did elicit a potentiated firing in the EUSE that, in turn, may induce and prolong EUS contraction (34, 35). On the other hand, when voiding occurred, the potentiated reflex activities subsided suddenly. This finding is consistent with Blok’s study (8). This demonstrated that the spinal micturition reflex activity of the pudendal motor neurons, located in Onuf’s nucleus, is inhibited by GABAergic and glycinegenic descending innervation arising from the pontine micturition center to facilitate urine emission. Finally, after a voiding contraction has finished, a neurally driven potentiation in reflex activities may close the urethra efficiently. All these findings suggested pelvic nerve-to-EUS reflex potentiations may be essential for urine continence in high-volume conditions during storage, for developing an efficient pressure gradient for urine emission during the ascending phase of voiding contractions and for urethral closing after a voiding has finished.

Randić et al. (43) suggested that tetanization-induced enhancement of excitatory postsynaptic potentials may be related to the mechanisms involved in the generation of postinjury pain hypersensitivity. Several researchers have reported several chemical irritation-induced hyperactivities in the urethral sphincter (16, 50). A high resistance in the lower urinary tract as a result of a hyperactive sphincter has been suggested to
cause obstructive bladder dysfunction (29). In the present study, we continuously infused the urinary bladder with an unblocked urethra that allowed periodical voids to occur. The means of the IVP, tested in this study, were generally within physiological ranges. However, to obtain the EUSE units of which activity can be clearly evaluated, those units with background discharges during the early stage of the storage period, i.e., one-fourth of the threshold IVP, were usually given up to avoid contamination of the evoked activity with background tonic discharges. Although the units we recorded were characterized by background discharges during the late stage of the storage period, i.e., higher than one-half of the threshold pressure, these units in the present study seem to be high-threshold ones. Therefore, whether these units mediated a pathological state of higher IVP should be seriously considered.

To maintain the afferent pathways for bladder distension to induce SRP in the pelvic nerve-to-EUS reflex activities, a bundle of intact pelvic nerves was stimulated instead of a central stump. The possibility of direct efferent impulses inducing modulations in EUSE activities can be ruled out for the following reasons. The reflex latency recorded in this study was 55.84 ± 2.57 ms, which was too long for the somatic pudendal nerve to elicit EUSE activities. Second, the pelvic efferent nerve innervates detrusor rather than the EUS, so EUSE activities are not affected by pelvic efferent fibers. Third, the stimulating parameter sufficient to induce a detrusor contraction via the postganglionic parasympathetic nerve is 5 Hz for 30 s or higher (31, 32). Single impulses with pulse durations of 0.1 ms that were used in this study are not able to elicit bladder contractions. Therefore, the possibility of firing, caused secondarily by detrusor contraction, is nearly impossible in the EUSE. Finally, at the end of the experiments, we stimulated the central stump of the pelvic nerve using the test stimulation and evoked an action potential with identical amplitude, shape, and time latency as was done with the intact nerve stimulations. The above-noted four reasons indicate that the activities recorded in this experiment are mainly the result of pelvic afferent inputs.

However, the detailed mechanism underlying the cyclic SRP during periodical micturition cycles is not yet clear. The induction of activity-dependent reflex potentiation is presently thought to activate glutamatergic NMDA and AMPA receptors (3, 18, 21, 24, 30, 40). We found that NMDA and AMPA receptor antagonists attenuated the reflex potentiation in the pelvic nerve-to-EUS reflex activities, suggesting that the cyclic SRP in the pelvic nerve-to-EUS reflex may share a similar mechanism to the long-term potentiation. This conjecture is in accordance with a recent report which has shown that the strength of primary afferent transmission might be potentiated following tetanic peripheral inputs (43). Due to the limitation in the multiple-fiber recording technique used in this study, further investigation of the synaptic efficacy on the dorsal horn within the spinal cord needs to be executed to clarify whether the SRP presented in this study is mediated by a “long-term potentiation-like” synaptic transmission.

Glutamate is a widely utilized neurotransmitter in the lumbo-sacral spinal level, at which point mediating the function of the lower urinary tract including the urinary bladder and urethra. Cystometric investigations have revealed that the activities of the EUS are sensitive to glutamatergic NMDA and AMPA neurotransmission (22, 23). Pharmacological studies suggested glutamate is not only essential for the physiological function of the lower urinary tract (49, 56–60, 62) but is also a possible therapeutic target in urinary dysfunctions resulted from spinal cord injury (62) and cerebral infarction (55). These findings are 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, indicating a potential role of glutamatergic agonist/antagonist in further investigations on the therapeutic agents for urinary tract dysfunctions.

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