Nicotine-activated descending facilitation on spinal NMDA-dependent reflex potentiation from pontine tegmentum in rats

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THE IMPORTANCE OF UNDERSTANDING the descending innervations coming from the brain stem to the spinal circuitry involved in the micturition function has been emphasized because it may offer the strategy for developing pharmacological therapy in micturition disorders (38). Researchers applied electric shocks to the pontine reticular formation to relate this site with micturition function and revealed that the pontine reticular formation plays an important role in descending control on the functions of the lower urinary tract (30, 31, 54, 55, 67). The cholinergic system in brain areas of the central nervous system regulates physiological functions, including micturition (18, 21, 40). Administration of cholinergic agonists to the pontine tegmentum affected urodynamic parameters in reflexive micturition cycles (31, 67). In addition, pharmacological blockade of the nicotinic cholinergic receptors (nAChR) in the pontine tegmentum using chlorisondamine, a nicotinic receptor antagonist, abolished the modulation exhibited by cholinergic agonists (40, 56), suggesting that activation of nAChR in the pontine tegmentum is involved in the descending modulation on the spinal circuitry mediating micturition functions (19, 52).

Activation of nicotinic receptor in the brain area enhances the release of neurotransmitter, including norepinephrine, dopamine, glutamate, acetylcholine, and serotonin (5-hydroxytryptamine; 5-HT) in the terminal of descending fiber at the spinal cord level (16, 36, 50, 57, 60, 75). 5-HT has been shown to play an important role in the descending control of reflexive micturition functions (9, 10, 17, 21, 39, 69). 5-HT receptors are widely distributed in the lumbosacral spinal cord, which is the origin of parasymptathetic outflow regulating the lower urinary tract (24, 53, 54, 73). Among 5-HT-receptor subtypes, the 5-HT1A receptor had been well investigated due to the early availability of a selective 5-HT1A receptor antagonist, N-[2-(4-[(2-methoxyphenyl)-1-piperazinyl]ethyl)-N-(2-pyridyl)cy clohexanecarboxamide trihydrochloride (WAY-100635; see Refs. 22, 23, 34, 69). Previous experiments have demonstrated that the activation of descending 5-HT1A systems modulates the spinal integrated voiding cycles in rats and suggested the importance of spinal 5-HT1A receptors in the descending control of the micturition function (39).

Activity-dependent reflex plasticity, for example, long-term potentiation (5, 6, 52) and windup phenomenon (46, 65, 78), has been widely studied in the central nervous system for their presumed relevance to memory (51) and hyperalgesia (58), respectively. Forms of reflex plasticity can be elicited by...
applying electric shocks (15) and by reagent injections/infusions to specific sites (33). On the other hand, an established/establishing reflex plasticity may be modified by genetic (64), pharmacological (32, 58), surgical (37, 61, 68), or behavioral manipulations (3, 59). However, few studies explored the possibility of modulating activity-dependent reflex plasticity by activating a specific nucleus, which sends neural projection to the site, where the reflex plasticity occurs. Such a study may mimic the physiological modulation on reflex plasticity and offer gateways to elucidate the physiological/pharmacological relevancies in it (2).

Using in vivo animal preparations, we demonstrated that low-frequency repetitive stimulations (RS) on the pelvic afferent nerve elicited a form of activity-dependent reflex plasticity, spinal reflex potentiation (SRP) in the pelvic-urethra reflex activities (12, 13, 41, 42, 43, 44, 45). Such in vivo preparations used in these studies maintained the whole neural network within the the central nervous system intact, thus offering an animal model to explore the possibility that activity-dependent reflex plasticity can be regulated by a specific nucleus that projects nerve fibers to the site where plasticity occurs. We recently explored the descending modulations on the reflex potentiation coming from the pontine reticular formation and reported that electric stimulation at the dorsolateral pontine tegmentum might activate the descending fiber to the spinal cord to facilitate SRP induction (11). In the present study, we further explored the possible neurotransmitter to activate this descending system by microinjecting nAChR agonists to the dorsolateral pontine tegmentum. We also identified whether spinal 5-HT1A receptor mediates such a descending innervation at the spinal cord level by intrathecal injection of 5-HT1A receptor antagonist.

MATERIALS AND METHODS

General preparations. Sixty-three adult female Wistar rats weighing 250–350 g were anesthetized with urethane (1.2 g/kg ip). The National Science Council in Taiwan approved the animal care and experimental protocol. 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 corneal reflexes of the rats and responses to a noxious stimulation to the paw were monitored during the experiment. After localization of the stimulation point at the posvarollis, the ligation of the urethra opening was cut, and the transurethral bladder cannula was withdrawn. The lower body of the rat was rotated to a supine position, and a midline abdominal incision was made to expose the pelvic viscera. Both ureters were ligated distally and cut proximally to the ligation sites. The proximal ends of the ureters were ligated freely into the abdominal cavity. Another wide-bore intravesical bladder cannula was tied in the apex of the bladder dome. The urinary bladder was drained free to rule out unnecessary afferent inputs. Abdominal skin flaps were tied to a metal frame to form a skin pool, and the abdominal cavity was filled with warm mineral oil. After all the experimental procedures were finished, the stimulation site was electrically injured by passing a direct current (8.4 to 9.4 mm; lateral, 1.0 to 1.5 mm; height, 6.5 to 7.0 mm from the bregma) in 0.25- or 0.5-mm steps using a micromanipulator (34). Electrical stimulation in the dorsolateral pontine tegmentum consisted of sequences of stimulation (1–15 V, 0.05 ms in pulse duration at 300 Hz, 5 ms train duration). The optimal sites for inducing an isovolumic contraction in the urinary bladder, with the largest amplitude (usually >15 mmH2O), were determined in each experiment. After localization of the stimulation point at the posvarollis, the ligation around the urethra opening was done to immobilize the transurethral cannula and avoid fluid leakage from the bladder through the urethra. For recording intravesicular pressure (IVP), we connected the transurethral bladder cannula to a pressure transducer (P23 ID; Gould-Statham, El Segundo, CA), and the IVP was continuously recorded on a computer system (MP30; Biopac, Santa Barbara, CA) through a preamplifier (7P1; Grass, Cleveland, OH). Saline was injected through the transurethral bladder cannula to determine the threshold volume for inducing rhythmic isovolumic contractions. After the threshold volume was obtained, small amounts of saline were withdrawn from the bladder until bladder contractions disappeared. The bladder volume was kept isovolumically below the threshold for inducing spontaneous bladder contractions during brain stem stimulation. The needle electrodes were then introduced stereotaxically in the lateral part of the dorsolateral pontine tegmentum (anterior-posterior, −8.4 to −9.4 mm; lateral, 1.0 to 1.5 mm; height, −6.5 to −7.0 mm from the bregma) in 0.25- or 0.5-mm steps using a micromanipulator (34). Electrical stimulation in the dorsolateral pontine tegmentum was made to elicit the pelvic visera. Both ureters were ligated distally and cut proximally to the ligation sites. The proximal ends of the ureters were ligated freely into the abdominal cavity. Another wide-bore intravesical bladder cannula was tied in the apex of the bladder dome. The urinary bladder was drained free to rule out unnecessary afferent inputs. Abdominal skin flaps were tied to a metal frame to form a skin pool, and the abdominal cavity was filled with warm mineral oil. After all the experimental procedures were finished, the stimulation site was electrically injured by passing a direct current through, and then the site was reconstructed with reference to the lesion mark. Experimental animals of which the stimulation site deviated by >1 mm from the target structure were excluded from statistical analysis.

Nerve dissection and stimulation. The left pelvic nerve was dissected carefully from the surrounding tissue and was transected distally, whereas the right pelvic nerve was left intact. The central stump of the left pelvic nerve was placed on a pair of stainless steel wire electrodes and was stimulated by an electric current of square wave pulses with pulse durations of 0.1 ms applied by a stimulator (S88; Grass) through a stimulus isolation unit (SIUS5B; Grass) and a constant current unit (CCU1A; Grass). Euler activities recording. Epoxy-coated copper wire (50 µm; Giken, Tokyo, Japan) electromyogram electrodes were placed in the periurethra area intra-abdominally. This procedure 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 in the spincter ~1–2 mm lateral to the urethra and then withdrawn, leaving the electromyogram wire embedded in the sphincter. The electromyogram activities were amplified 20,000-fold, filtered (high frequency cut-off at 3,000 Hz and low at 30 Hz) by a preamplifier (PS11AC; Grass), and then continuously displayed on an oscilloscope (TDS...
spinal cord transection. In some experiments, the vertebrae were exposed along the cervicothoracic level and followed by a 4-cm laminectomy. After a pair of microscissor had carefully opened the dura, the spinal cord was transected at the T1 level using a microforceps.

**Experimental arrangement and protocol.** The schematic arrangement of reflex activity recordings, in response to the afferent nerve stimulation and pons varolli stimulation/injection, is shown in Fig. 1A. After the urinary bladder drained free via the intravesical bladder cannula, the pelvic afferent nerve was stimulated using the parameters as follows: the test stimulation (TS) was single shocks at fixed suprathreshold strengths with a frequency of 1/30 Hz and lasting for 10 min. The RS was single shocks with a frequency of 1 Hz also lasting for 10 min with an intensity identical to the TS. Train pontine stimulation was train pulses synchronized with afferent fiber RS (1 Hz) with 300 Hz in pulse frequency and 5-ms train duration.

**Spinal cord transection.** In some experiments, after facilitation on RS-induced SRP had been established by pontine nicotine microinjection and the animal has been connected to a ventilator, the spinal cord was transected at the T1 level to verify the pathway involved in facilitation on SRP.

**Application of drugs.** Drugs were administered by intrathecal injections, including 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo (F) quinoxaline (NBQX, a glutamatergic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) receptor antagonist, 100 μM, 10 μl; Sigma), d-2-amino-5-phosphonovaleric acid (APV, a glutamatergic N-methyl-D-aspartic acid (NMDA) receptor antagonist, 100 μM, 10 μl; Sigma), L-glutamate (a nonselective glutamatergic receptor agonist, 100 μM, 10 μl; Sigma), NMDA (a glutamatergic NMDA receptor agonist, 100 μM, 10 μl; Sigma), WAY-100635 (a selective

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**Fig. 1. Effects of pelvic afferent nerve stimulation and dorsolateral pontine tegmentum train stimulations.** A: schematic arrangement of the external urethra sphincter electromyogram (EUSE) recording in response to pelvic afferent nerve and pontine stimulations or injections. VC, visual cortex; IC, inferior colliculi; PY, pyramidal tract. The black circle indicates the range of the locations of the stimulation/injection electrode tips. B: short train pulse pontine stimulation (PS) produced an increase in intravesical pressure (IVP) in a isovolumically filled urinary bladder[PS + distension (DIS)]. C: after the urinary bladder was drained free, a short train pulse pontine stimulation (PS) evoked no action potential in the electromyogram activity. D: after the urinary bladder was drained free, a single action potential in the reflex activity was induced by pelvic afferent nerve test stimulation (TS).
serotonergic 5-HT₁A receptor antagonist, 100 μM, 10 μl; Sigma), ACh (a nonselective nAChR agonist, 100 μM, 10 μl; Sigma), (+)nicotine (a selective nAChR agonist, 100 μM, 10 μl; Sigma), and cholorisondamine (a selective nAChR antagonist, 100 μM, 10 μl; Sigma). All drugs were dissolved in artificial cerebrospinal fluid (in mM: 118 NaCl, 3 KCl, 25 NaHCO₃, 12 NaH₂PO₄, 1 MgCl₂, 1.5 CaCl₂, and 10 glucose; pH = 7.4) or dimethyl sulfoxide (DMSO) and applied in final DMSO concentration <1%. The solution of identical volume to tested agents was dispensed to serve as the vehicle. This selected dose of drug was used because previous studies showed that glutamate and NMDA could induce pelvic-pudendal reflex plasticity in urethane-anesthetized rats at this dose (44, 45). Besides, previous studies showed that 5-HT₁A receptor antagonist may modulate micropuncture reflex at the spinal cord level (20, 25, 26). Therefore, we used the above tested agents and adopted this effective dose. The dose-response curve was not obtained in our study.

Data analysis. All data in the text and Figs. 1–4 are mean values ± SE. Statistical analysis of the reflex excitability between groups over the stimulation period was performed by means of ANOVA followed by a post hoc test. In all cases, a difference of P < 0.05 was considered to be statistically significant.

RESULTS

Complete data were obtained from 63 rats. Under some conditions, animals were excluded from analysis, including three rats did not complete the experimental procedures; in two rats the stimulation/injection electrode deviated by >1 mm, and in two rats the cannula tip for intathecal injections deviated by >0.5 mm from the target structure. Therefore, there were 56 animals for statistical analysis. TS, RS, glutamatergic agonists/antagonists, and synchronized pontine stimulation were tested in all animals. There were 10 rats for spinal transection after pontine stimulation, 10 rats for pontine ACh injections, and 30 for pontine nicotine injections. In rats receiving pontine nicotine injections, 10 rats received pretreatment of cholorisondamine, 10 rats were further tested by intrathecal serotonergic antagonist injections, and the other 10 rats received spinal cord transection. In addition, there are an extra three rats each in glutamate agonist/antagonist and nicotine injection for vehicle control.

Pontine electrical stimulation. As shown in Fig. 1B, after saline of subthreshold volume has been instilled in the urinary bladder via the transurethral bladder cannula, pontine train stimulation produced a contraction wave in the isovolumically filled urinary bladder, indicating that the location of the electrodes was optimum. Next, the transurethra cannula was withdrawn. A midline abdominal incision exposed the urinary bladder and urethra, and an intravesical bladder cannula was inserted in the urinary bladder from the bladder dome so that the urinary bladder could drain freely. Train stimulation at the same site with identical parameters, after the bladder has drained freely, evoked no action potential in the external urethra sphincter electromyogram (Fig. 1C).

Baseline reflex activities. The reflex activities evoked by the pelvic afferent nerve TS (1/30 Hz) after the urinary bladder has drained freely, obtained from one of the 40 rats, are shown in Fig. 1D. A baseline reflex activity with a single action potential was elicited by the TS. The mean latency for the TS to evoke the activity was 57.42 ± 0.31 ms (n = 40).

RS-induced reflex potentiation. As shown in Fig. 2A, a longer-lasting reflex potentiation was induced by the afferent nerve RS (1 Hz) with the same intensity as the TS. The evoked activity increased gradually following the onset of the RS, reached a plateau at ~2 min, and maintained this level until the cessation of stimulation. Summarized data in Fig. 2B show that the mean spike numbers evoked by the RS counted 10 min following stimulation onset increased significantly compared with the baseline reflex activities induced by the TS (P < 0.01, n = 40).

Glutamatergic agonists and antagonists. As shown in Fig. 2A, intrathecal administration of glutamate and NMDA both induced longer-lasting potentiation in the TS-induced reflex activities that is similar to that induced by the RS. On the other hand, intrathecal NBQX attenuated and intrathecal APV blocked the RS-induced reflex potentiation. Vehicle injections caused no effect on the test/RS-elicited reflex activity; therefore, the data were pooled to the TS/RS alone. The summarized data in Fig. 2B show that intrathecal glutamate and NMDA significantly increased the spike number evoked by the TS when compared with the TS (P < 0.01, n = 40). Meanwhile, intrathecal NBQX and APV significantly decreased the spike number evoked by the RS when compared with RS alone (P < 0.01, n = 40).

Synchronized pontine electrical stimulation. As shown in Fig. 3A, repetitive train pontine stimulation (1 Hz) evoked no action potential in the pelvic-urethra reflex activities. However, synchronized train pontine stimulation with the repetitive afferent nerve stimulation exaggerated the reflex potentiation compared with that elicited by afferent nerve RS alone. A spinal cord transection at the T₁ level abolished the facilitation caused by synchronized pontine stimulation but did not affect the reflex potentiation caused by pelvic afferent nerve RS. The summarized data in Fig. 3B show that the spike number elicited by the repetitive afferent fiber stimulation with the synchronized pontine stimulation was significantly higher than that induced by the repetitive afferent fiber stimulation alone (P < 0.01, n = 40). In addition, the facilitation on the RS-induced reflex potentiation caused by synchronized pontine stimulation was abolished by the spinal transection (P < 0.01, n = 10).

Effects of nAChR agonists. As shown in Fig. 4A, microinjection of ACh, a nonselective nAChR agonist, to the dorsolateral pontine tegmentum facilitated the RS-induced reflex potentiation that is similar to the synchronized pontine electrical stimulation. On the other hand, a selective nAChR agonist, nicotine, also produced facilitation on the RS-induced reflex potentiation that is similar to that induced by the RS. The summarized data in Fig. 4B show that nicotine significantly increased the spike number evoked by the RS when compared with RS alone (P < 0.01, n = 40). Meanwhile, intrathecal NBQX and APV significantly decreased the spike number evoked by the RS when compared with RS alone (P < 0.01, n = 40).

Effects of 5-HT antagonist. Intrathecal pretreatment of WAY-100635, a 5-HT₁A receptor antagonist, did not affect the baseline reflex activity evoked by the TS (data not shown); however, as shown in Fig. 4A, it abolished the facilitation elicited by the pontine nicotine injection. Vehicle injections caused no effect on the RS-induced reflex potentiation; therefore, the data were pooled to the RS alone. The summarized data in Fig. 4B shows that WAY-100635 significantly de-
creased the spike number caused by RS in association with pontine nicotine microinjection when compared with RS with pontine nicotine injection (P < 0.01, n = 10).

Effect of spinal cord transection. As shown in Fig. 4A, a spinal cord transection at the T1 level abolished the facilitation exhibited by nicotine injection. The summarized data in Fig. 4B show that spinalization at the T1 level significantly decreased the spike number evoked by RS in association with pontine nicotine microinjection when compared with RS in association with pontine nicotine microinjection (P < 0.01, n = 10).

DISCUSSION

Under physiological conditions, urine distension activates stretch receptors on the bladder wall and therefore generates action potentials, which propagate along the pelvic afferent nerve in the spinal dorsal horn. These impulses ascend via
interneurons to activate the pontine micturition center. If micturition is signaled by the center as appropriate, descending axons coming from this brain center activate sacral parasympathetic preganglionic neurons that traverse the pelvic efferent nerve and subsequently cause contraction in the bladder detrusor and relaxation in the urethra sphincter to induce voiding.

On the other hand, a more rapid, somatic storage reflex, the guarding reflex, is initiated in response to a sudden increase in bladder pressure, such as during a cough, laugh, or sneeze. In the guarding reflex, afferent impulses caused by an abrupt increase in bladder pressure conduct through the pelvic afferent nerve to the sacral spinal cord, and activate the striated urethral muscle via interneurons to sphincter motor neurons in the Onuf nucleus, thereby “guarding” against urine leakage during a sudden unexpected increase in bladder pressure.

In the present study, train pulses stimulation at the dorsolateral pontine tegmentum produces detrusor contraction in the isovolumic filled bladder. Moreover, synchronized pontine stimulation to the pelvic afferent stimulation exhibited an augmentation in the RS-induced SRP. However, the relevance of the facilitation on SRP caused by the dorsolateral pontine tegmentum descending projections has not yet been elucidated. We suggested that the descending projections coming from the dorsolateral pontine tegmentum might facilitate the guarding reflex activity, which is characterized by a urethra contraction caused by a sudden bladder pressure increase, and therefore promotes the reflexive urethra closure to avoid urine leakage during an abrupt bladder pressure increase. This result is quite correlated with urodynamic investigations that suggested the pontine micturition center may regulate the spinal cord circuitry controlling micturition functions via descending pathways in normal (31, 40) and stroke (80) rats. On the other hand, in this study, pharmacological activation of dorsolateral pontine tegmentum by microinjection of nAChR agonists exhibited descending facilitation on the RS-induced SRP. This result implied that the descending cholinergic pathway coming from the

Fig. 3. Synchronized train pontine stimulation facilitated the RS-induced reflex potentiation. A: when compared with a baseline reflex activity with single action potentials in the EUSE elicited by the pelvic nerve afferent nerve TS, a long-lasting reflex potentiation was induced by the RS. Although the repetitive train pontine stimulation (PS) evoked no action potential, synchronized train pontine stimulation with pelvic afferent nerve RS facilitated the RS-induced reflex potentiation (PS + RS). A spinal cord transsection at the T1 level abolished the facilitation on the RS-induced reflex potentiation caused by synchronized train pontine stimulation (RS + PS + SCX). The tracings before and after the break symbol show the reflex activities at the onset of stimulation and at 10 min following the stimulation onset. B: mean spike nos. (mean ± SE, n = 10) induced by the pelvic afferent nerve stimulation with or without synchronized pontine stimulation. "P < 0.01, significantly different from activities induced by TS (**), RS (#), and RS in association with pontine stimulation (++)".

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brain area plays a role in regulation of guarding of urine from leakage, and nAChR seems to be a potential candidate for developing strategies in treatment of stress urine incontinence caused by urethra insufficiency resulting from neurological origins. This proposal is in agreement with pharmacological investigations in which intracereboventricular nicotine injection increased the intercontraction interval in a continuously infused cystometry (29). However, there is a concern in this study about the possibility that, although the bladder was maintained empty during the stimulation period, pontine stimulation may induce undetectable bladder contraction, which increased the afferent impulse through the intact nerve trunk contralaterally to the stimulation, and result in potentiation in reflex activity. This possibility should be seriously considered in future investigations.

Serotonergic 5-HT_{1A} receptors have been identified at the lumbosacral spinal cord, where preganglionic neurons innervating the lower urinary tract are located (54, 73). Two functionally distinct 5-HT_{1A} receptor populations, somatodendritic autoreceptors and postsynaptic receptors, mediate serotonergic neurotransmission within the spinal cord. Several 5-HT_{1A} receptor partial agonists have been identified that act as antagonists at postsynaptic receptors and agonists at presynaptic receptors (23). However, this combination of effects of 5-HT_{1A} receptor partial agonists causes complex and conflicting pharmacological results. Forster et al. (23) and Fornal et al. (22) found WAY-100635 to be a potent, selective, and silent 5-HT_{1A} receptor antagonist; therefore, this compound was widely used in pharmacological investigations on serotonergic neurotransmission. In the present study, we used WAY-100635 to evaluate the role of 5-HT_{1A} receptors in the regulation of micturition function a the spinal cord level. Our results showed that the descending facilitation on the SRP caused by pontine electric stimulation and nACHR agonists was abolished by the
pharmacological blockade using WAY-100635 at the spinal cord level, indicating that spinal 5-HT$_{1A}$ neurotransmission at the lumbosacral spinal cord mediates the descending projections from the dorsolateral pontine tegmentum to regulates reflexive micturition function. This result is consistent with previous reports that the 5-HT$_{1A}$ receptor antagonist abolished descending facilitation caused by electric stimulation at the dorsolateral pontine tegmentum. Moreover, such an abolition was reversed by the pretreatment of 5-HT$_{1A}$ receptor agonist, indicating an excitatory role of spinal 5-HT$_{1A}$ receptors in the descending control of the lumbosacral spinal reflex activity (7, 8, 25, 26, 40, 39, 70, 71). However, the mechanism by which 5-HT affects the glutamatergic NMDA-dependent SRP presented in this study is still waiting for elucidation. In vitro electrophysiological studies recording the dorsal horn field potential (47) and whole cell patch-clamp recording (62) have demonstrated that 5-HT may facilitate C fiber-evoked excitatory postsynaptic potential in a subpopulation of lamina III-VI spinal neurons. However, the detailed intracellular cascade involved and the physiological relevance needs further investigations.

Except being essential for urethral closure, pathological overactivation of the neural substrate involved in the pelvic-urethra reflex activity caused by injury or inflammation may produce urethra hyperreflexia or detrusor-sphincter dys-synergia (28), which are seen in patients with lower urinary tract syndrome (74, 79). Therefore, whether the blockage of descending facilitation on the SRP and urethra activity caused by WAY-100635 also implied that spinal serotonergic 5-HT$_{1A}$ receptors can be substantial candidates to relieve the neuropathic hyperactive or dys-synergic urethra in patients with lower urinary tract syndrome (20, 72) is an interesting topic to be investigated further.

A repetitive activation of synaptic connections leads to activity-dependent reflex plasticity in brain areas (33, 48, 63). Forms of modulation on activity-dependent reflex plasticity, including long-term potentiation (1, 4) and central sensitization (76, 77), are still being examined because they may offer a gateway to clarify the physiological relevance and the possibility of pharmacological manipulation in activity-dependent neural responses (3, 61). It is widely accepted that glutamatergic NMDA neurotransmission underlies activity-dependent reflex plasticity in the central nervous system (66). Investigations on the neural control of the lower urinary tract, including the urinary bladder (81, 82, 84) and urethra (10, 83), have revealed the role of glutamatergic NMDA-dependent neurotransmission in the spinal-mediated micturition functions. In this study, we pharmacologically blocked spinal glutamatergic neurotransmission by intrathecal NBQX and APV injections and demonstrated that a glutamatergic NMDA-dependent neurotransmission was involved in the RS-induced SRP. These findings suggest that RS-induced SRP may share a similar glutamatergic NMDA-dependent neurotransmission to the well-investigated long-term potentiation (14, 27, 35, 49). However, there are some obvious differences between long-term potentiation and the SRP present in this study. First, LTP is induced by high-frequency tetanic afferent input that is usually beyond 100 Hz, whereas a low-frequency stimulation paradigm (of 1 Hz) was used in this study. In addition, LTP lasts for hours after tetanization, whereas SRP decays shortly after RS cessation. In our unpublished data, the evoked activity usually recovered to the baseline level within 1 min, even if the afferent fiber was continuously stimulated with the TS following the RS offset. Furthermore, LTP applies to an increment in a single synaptic efficacy that is typically measured by a synaptic potential rather than action potentials, which were recorded in this study to reflect a reflex activity, and it is not a monosynaptic event. Therefore, we suggest that SPR, which was facilitated by descending serotonergic innervations in the present study, is possibly not an “LTP-like” synaptic transmission.

Recently, using in vivo animal preparations with their central nervous system intact, our laboratory has demonstrated that applying train pulses to the dorsolateral pontine tegmentum might activate serotonergic descending pathways to modulate the SRP, a form of activity-dependent reflex plasticity. This result implied that an established/establishing reflex plasticity could be modulated by a specific nucleus, which sends projections to the site where the plasticity occurs, and offered a novel model to investigate the neural regulation of plasticity induction. In this study, we further extended our work by activating the dorsolateral pontine tegmentum using nAChR agonists, and our data showed that microinjection of ACh and nicotine to the dorsolateral pontine tegmentum both facilitated the RS-induced SRP. Moreover, after nAChR agonist has established facilitation on SRP, a high thoracic spinal cord transection abolished the facilitation caused by reagent injection. These data showed that descending regulation on the induction of activity-dependent reflex plasticity at the spinal cord level can be elicited by activating a specific nucleus using a widely utilized neurotransmitter within the central nervous system, i.e., implies that modulation of the induction of neural plasticity from another neural nucleus cannot only be elicited by using nonphysiological electric shock on a neuron but can also be done by activating the receptors on such a nucleus using physiological neurotransmitters. This in vivo animal preparation offers a model for investigating the regulation on the induction of plasticity using physiological neurotransmitter and for developing pharmacological therapeutic strategies on modulation of plasticity.

In summary, our findings in this study demonstrated that single-pulse pelvic afferent nerve stimulation evoked a baseline pelvic-urethra reflex activity, whereas RSs elicited long-lasting SRP, which can be facilitated by synchronized pontine stimulation. Pharmacological activation of nAChR at the dorsolateral pontine tegmentum by ACh and nicotine microinjections produces facilitation on the RS-induced SRP in a manner similar to the synchronized pontine stimulation, indicating involvement of nAChR in the activation of the descending projections coming from the dorsolateral pontine tegmentum to modulate the induction of activity-dependent neural plasticity at the spinal cord level. In addition, pharmacological blockade of serotonergic 5-HT$_{1A}$ receptor at the spinal cord level and spinal cord transection both abolished the facilitation caused by nicotine injection at the dorsolateral pontine tegmentum, implying that spinal serotonergic 5-HT$_{1A}$ receptor could be a potential candidate for developing pharmacological strategies in treatment of patients with neurological urethra hyperexcitability or dys-synergia.

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