Pelvic-Urethra Reflex Potentiation

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ACTIVITY-DEPENDENT NEURAL PLASTICITY

The efficacy of synaptic transmission is not a constant, but could vary depending on the neuronal circuitry within the nervous system as well as patterns of ongoing activities [1]. Repetitive activation of synaptic connections leads to modulation of synaptic efficacy in a variety of brain structures [2-4]. Long-term potentiation (LTP) [5,6] is characterized by a long-lasting enhancement of the efficacy of the excitatory synapse following a strong tetanic stimulation of input fibers. LTP in the CA1 area of the hippocampus has been investigated extensively in the past three decades because it is considered a fundamental mechanism of learning and memory formation [4,7]. In addition, another type of activity-dependent synaptic plasticity, termed "windup", is a progressive increase in evoked activity which occurs in the dorsal horn neurons of the spinal cord under repetitive low frequency nociceptive input. The windup phenomenon has been presumed to underlie the development of allodynia and/or hyperalgesia [8,9].

GLUTAMATERGIC NEUROTRANSMISSION

Glutamate is widely utilized in the spinal cord for primary afferent neurotransmission [10]. The glutamatergic receptors can be classified as metabotropic and ionotropic receptors. Metabotropic glutamatergic receptors that couple to G-proteins may initiate intracellular messengers. On the other hand, ionotropic glutamatergic receptors are classified into three subtypes, including N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionat (AMPA)/quisqualate and kainate receptors. Ionotropic glutamatergic receptors directly regulate permeability to sodium and potassium ions, and in the case of NMDA receptors, calcium ions as well [11]. Activation of both iontropic and metabotropic receptors can result in the elevation of the intracellular free calcium ion concentration, and in turn, lead to activation of secondary messenger systems with consequent changes in the properties of receptor channel complexes that contribute to long-term influences on excitatory synaptic transmission [12]. Studies investigating neural plasticity in the hippocampal CA1 area suggested that activation of glutamatergic NMDA and AMPA receptors mediates the induction of LTP [13]. Similar glutamate NMDA/AMPA receptor-dependent synaptic plasticity was also demonstrated in the dorsal horn of spinal cord preparations [10].

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PELVIC-URETHRA REFLEX POTENTIATION

Storing urine and voiding periodically are important functions of the lower urinary tract. During the storage phase of the micturition cycle, sensory impulses induced by bladder distension transmit centripetally onto the dorsal horn neurons through the pelvic afferent fibers [14]. After integrating within the spinal cord, motor impulses emerge via the pudendal efferent fibers, and therefore, cause external urethra sphincter contractions [15,16]. This pelvic-urethral reflex (PUR) is essential for the urethra to develop sufficient resistance to maintain continence [17]. Neural plasticity of PUR activity was first reported in a study that demonstrated the number of action potentials in pudendal efferent nerves and external urethra sphincter electromyogram activity evoked by pelvic afferent nerve (PAN) repetitive stimulation (RS, 1 stimulation/1sec) increased progressively, then reached a plateau which was maintained until stimulation ceased. In contrast, PAN test stimulation (TS, 1 stimulation/30 sec) evoked relatively constant baseline reflex activity with a single action potential [18].

PHYSIOLOGICAL RELEVANCE

Whether stimulation-induced PUR potentiation could affect the physiological function of the urinary tract was tested by recording intraurethral pressure, which reflects urethral contraction. Results of these studies showed PAN RS simultaneously elongated the contraction wave of the urethra in parallel with induction of PUR potentiation [18-20], indicating the electrical impulse-evoked reflex potentiation did modulate lower urinary tract function. Next, instead of electrical shock, bladder saline distention, an adequate natural stimulation of PAN fibers, was used to induce PUR potentiation. Stepwise saline distension of the urinary bladder from 0 to 4, 8, 12 and 16 mmHg dose-dependently potentiated TS-evoked baseline PUR activity accompanied by elongation of the urethra contraction wave [21], implying PUR potentiation could be induced by physiological bladder distension. Finally, a cystometrical study in rats tested whether PUR potentiation could be induced during the micturition cycle. During the early storage stage, negligible increments in intravesical pressure did not induce background spontaneous firing in urethra electromyogram activity, whereas, off-line analysis demonstrated that TS-evoked baseline PUR activities were potentiated in parallel with intravesical pressure increases during this stage [21]. This result implies the strength of the PUR fluctuates following the micturition cycle under physiological urinary functions. Moreover, PUR potentiation could be a physiological phenomenon that occurs under physiological conditions.

SPINAL NEUROTRANSMISSION

Glutamatergic NMDA and AMPA receptors

Pharmacological investigations using intrathecal injections demonstrated that PUR potentiation caused by repetitive electric shocks [22-25], bladder saline distension [21], rhythmic voiding cycle [26] and noxious visceral irritation [27-35] is abolished by intrathecal application of 2-amino-5-phosphono-valerate (APV), a glutamatergic NMDA receptor antagonist, and attenuated by 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX), a glutamatergic AMPA receptor antagonist. Conversely, spinal administration of NMDA and AMPA both provoke reflex potentiation in TS-induced baseline reflex activity [27-34], indicating roles of spinal NMDA and AMPA receptor subtypes in the induction of PUR potentiation.

NMDA receptor NR2B subunit phosphorylation

NMDA receptor (NMDAR) NR2B subunit, as a major unit controlling NMDAR activity via phosphorylation mechanisms, was found to play a pivotal role in the NMDA-dependent form of neural plasticity at the spinal cord level [36-39].

Accompanied by RS-induced PUR potentiation, a parallel phosphorylation of the NR2B subunit in the lumbosacral (L6-S2) dorsal horn ipsilateral to the stimulation was revealed by Western blot analysis. Pharmacological blockage of NR2B phosphorylation at the spinal cord level antagonizes reflex potentiation [24,29,31,32,34,35,40]. Finally, small interfering RNA (siRNA) of NR2B abolishes NR2B expression in association with preventing PUR potentiation [29,31,32,34,41] suggesting NMDAR NR2B phosphorylation at the lumbosacral spinal cord level is involved in PUR potentiation.

AMPA receptor GluR subunit trafficking

Intracellular redistribution of AMPA receptors (AMPAR) is known to underlie several forms of neural plasticity [42]. Agonist-induced PUR potentiation has been shown to provoke simultaneous AMPAR GluR1 subunit trafficking from the cytoplasm to the plasma membrane of lumbosacral spinal dorsal horn neurons [27], suggesting a participation of AMAPR trafficking in PUR potentiation.

DESCENDING CONTROL

The importance of understanding the descending innervations from the brain stem to the spinal circuitry involved in micturition function has been emphasized because it may offer strategies for developing pharmacological therapies for micturition disorders. Researchers applied electric shocks to the pontine reticular formation and revealed that it plays an important role in descending control on urinary tract functions [22,43]. In the pons varolli, the cholinergic system has been implicated in regulating micturition [1,17] because microinjection of cholinergic agonists to the pontine tegmentum affected urodynamic parameters in reflexive micturition cycles [22,44], and conversely, pharmacological blockage of the nicotinic cholinergic receptors (nACh) in this area abolished the modulation exhibited by cholinergic agonists [45,46]. On the other hand, the role of spinal serotonin (5-hydroxytryptamine; 5-HT) neurotransmission, which is widely distributed in the origin of parasympathetic outflow in the lumbosacral spinal cord [47-49], has been intensively investigated, and studies have revealed a crucial role of spinal 5-HT1A receptors in descending control of spinal reflex activities [50-52]. Microinjections of acetylcholine (ACh) and nicotine to the dorsal pontine tegmentum (DPT) both produced facilitation of RS-induced PUR potentiation. Pretreatment with selective nicotinic receptor antagonist at the DPT and spinal cord transection at the T1 level both abolished the nicotine-dependent facilitation of reflex potentiation [53], suggesting that activating of nicotinic ACh receptors at the DPT could modulate reflex potentiation via descending pathways to the spinal cord level. On the other hand, intrathecal administration of WAY 100635, a selective 5-HT1A antagonist, reversed the facilitation of PUR potentiation caused by DPT nicotine injection [53,54]. Synchronized train pulse DPT stimulation with RS facilitated RS-induced PUR potentiation was also reversed by intrathecal WAY 100635 adminstration. Intrathecal 8-OH-DPAT, a selective 5-HT1A agonist, elicited facilitation of reflex potentiation without synchronized potine electric stimulation, implying the DPT may facilitate the PUR via descending serotonergic neurotransmission [54]. Together these data suggest that activating of nicotinic ACh receptors at the DPT may modulate NMDA-dependent reflex potentiation via descending serotonergic neurotransmission.

IMPACTS OF GONADAL HORMONES

Estrogen

Estrogen is a gonadal steroid with pronounced tropic effects on neurons. Estradiol administration increases the synaptic excitation of pyramidal cells in the CA1 area. Surgical ablation of menses decreases the duration of the excitatory postsynaptic potential in the hippocampus [7,55,56]. Moreover, synaptic plasticity in the CA1 area is modulated by the menstrual cycle [7,57] and hormone replacement therapy [58]. Together these studies suggest that estrogen impacts reflex plasticity. Whether levels of circulatory estrogen affect lower urinary function through effects on PUR potentiation, a spinal form of neural plasticity, was first investigated using rats which received a sham operation (Sham), ovariectomy (OVX), or ovariectomy followed by estrogen supplementation (OVX+E). The magnitude of the RS-induced PUR potentiation and urethra contraction wave elongation decreased significantly in the OVX group, which was reversed by supplemental estrogen [19], indicating estrogen impacts lower urinary function through modulating PUR potentiation. The role of estrogen on PUR potentiation was further investigated by recording the evoked reflex activity in rats in different estrus stages of the female cycle. Noxious visceral stimulation induced PUR potentiation in both the proestrus and metestrus stages, and the degree of reflex potentiation was significantly higher in the proestrus rats than the metestrus ones [35]. These results imply the strength of the PUR could fluctuate in response to estrogen levels across different estrus stages.

In addition to genomic actions mediated by nucleus receptors, estrogen has non-genomic actions that lead to pathways that participate in acute responses via estrogen receptor-integrated activation of signal transduction cascades [59,60]. Through their effects on membrane receptors, gonadal hormones have been demonstrated to affect induction of pain-related LTP and reflex potentiation at the spinal cord level [19-20,23,30,35,41]. Administration of 17β-estradiol (5 μg/kg) was demonstrated to acutely facilitate noxious visceral stimulation-induced PUR potentiation that was reversed by intrathecal pretreatment with ICI 182780, a non-selective membrane estrogen receptor antagonist [28], indicating a role of membrane estrogen receptor

on the estrogen-dependent facilitation of PUR potentiation.

Progesterone

In addition to estrogen, progesterone is a gonadosteroid known to exhibit broad effects on the urogenital system. A regimen of daily progesterone for four days attenuated RS-induced PUR potentiation and simultaneously regulated the expression of $GABA_A$ receptor alpha 2, alpha 3, alpha 4 and delta subunits in ovariectomized rats. Finasteride, an antagonist of neurosteroid synthesis from progesterone, but not RU486, a progesterone receptor antagonist, reversed the progesterone-dependent inhibition of reflex potentiation. Moreover, PUR potentiation was attenuated after a short intrathecal treatment with the neurosteroids, allopregnanolone and 3α,5α-tetrahydrodeoxycorticosterone (THDOC). Acute intrathecal administration of the $GABA_A$ receptor antagonist bicuculline reversed the inhibition produced by progesterone, THDOC and allopregnanolone. These results imply that through its metabolic neurosteroid, progesterone inhibits PUR potentiation by exerting effects on spinal $GABA_A$ receptor expression [31]. This proposal is further supported by a study that showed progesterone as well as two of its 3α,5α-derivatives, allopregnalonone and THDOC, is capable of producing acute $GABA_A$ receptor-dependent inhibition of PUR potentiation [61].

CLINICAL APPLICATION

Rrelief of urethral spasms

To investigate the rationale that anal stretch could relieve the high urethral resistance in neurogenic bladder or detrusor-sphincter dyssynergia, the impact of acute anal stretch on PUR potentiation was examined in urethane-anesthetized rats. Acute anal stretch using a mosquito clamp with a distance of 4 mm exhibited no effect, whereas distances of 8 mm attenuated and 12 mm abolished the RS-induced spinal reflex potentiation (SRP). Intrathecal pretreatment with bicuculline, a $GABA_A$ receptor antagonist, but not hydroxysaclofen, a $GABA_A$ receptor antagonist, counteracted the anal stretch-dependent abolition of PUR potentiation [62]. These results suggested that GABAergic neurotransmission is an important spinal mechanism involved in inhibition of reflexive external urethra sphincter activity. Moreover, acute manual anal stretch, a maneuver that activates the spinal GABAergic system, may be used as an adjunct to assist voiding dysfunction in patients with overactive urethral sphincters.

Tacrolimus

Tacrolimus (FK506), a macrolide compound isolated from *Streptomyces tsukubaensis*, has potent immunosuppressive properties [63], and therefore, has been widely used in organ transplantation to prevent allograft rejection [64]. One of its side effects is neurotoxicity [64]. In addition, FK506 was shown to have inhibitory effects on glutamatergic neurotransmission [65-67]. In naive rat preparations, acute intrathecal tacrolimus blocked RS-induced PUR potentiation that was reversed by glutamate NMDA [68]. This finding may be pathologically relevant and investigations should determine if tacrolimus induces urinary incontinence in patients who take it for immunosuppressant therapy

Cyclophospamide

Because of the urotoxicity of its metabolite acrolein, patients that receive cyclophosphamide (CYP) as an anti-neoplasmic agent are known to develop interstitial cystitis, characterized by hematuria [69] and suprapubic pain [70]. *In vivo* animal studies have demonstrated that intra-peritoneal CYP administration induces cystitis symptoms such as irritative voiding, gross hematuria, and lower abdominal pain that resemble interstitial cystitis [71]. Therefore, CYP-induced cystitis has been used as an animal model for bladder-related pain and inflammation [71-73]. The impact of chronic bladder pain and inflammation on PUR potentiation relevant to urethral closure has been investigated in rats treated with CYP. Compared with vehicle solution, CYP increased the evoked spikes in PUR potentiation which was prevented by intraperitoneal pretreatments with L-NAME, a NO synthetase inhibitor, and roscovitine, a Cdk5 antagonist [40]. These results suggested that the chronic bladder inflammation caused by CYP might facilitate SRP, and therefore, participate in the development of urethral hypereflexia through NO- and Cdk5-dependent pathways.

D2R agonists

Descending innervation to the spinal cord exhibits complex involvement in micturition, including both inhibition and facilitation [17]. Among the neurotransmission pathways involved in descending modulation, the role of the dopaminergic D2-like receptors (DR2) in micturition has been investigated in patients with Parkinson's disease (PD) [74]. Cystometric studies have demonstrated that DR2 activation induces detrusor overactivity in rats [75,76]. Clinical trials have also shown that levodopa treatment exacerbates detrusor overactivity, urgency, and urge incontinence in PD patients [77]. In addition, PD patients who receive bromocriptine, a selective DR2 agonist, exhibit urinary frequency and urgency [15]. In addition to overactive bladder syndrome, PD patients exhibit mild urethral hyperactivity characterized by delayed or incomplete urethral relaxation during voiding [74]. A recent study of intravesical and intraurethral pressures demonstrated that both systemic and intracerebroventricular administration of a DR2 agonist reduced baseline urethral pressure by suppressing the striated urethral sphincter. The impacts of spinal dopaminergic DR2 on PUR potentiation were tested using *in vivo* rat preparations, and results demonstrated that intrathecal application of quinelorane dihydrochloride, a selective DR2 agonist, dose-dependently inhibited RS-induced PUR potentiation. Conversely, pretreatment with L135, a selective DR2 antagonist, antagonized Q110-dependent inhibition [25], suggesting DR2 activation at the lumbosacral spinal dorsal horn could inhibit PUR potentiation that is thought to be involved in reflexive urethral closure.

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