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Roles of Glycinergic and Gamma-aminobutyric-ergic Mechanisms in the Micturition Reflex in Rats

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Abstract

The micturition reflex is one of the autonomic reflexes mediated by the spinobulbospinal reflex pathway that passes through the pontine micturition center. In the central nervous system, glutamate is a major excitatory amino acid, while glycine and gamma-aminobutyric acid (GABA) are major inhibitory neurotransmitters and act to inhibit the micturition reflex at supraspinal and/or spinal sites. Glycine and GABA have additive or synergistic inhibitory effects on bladder activity. Hypofunction of glycinergic/GABAergic mechanisms in the lumbosacral spinal cord induces voiding dysfunctions, such as detrusor overactivity (DO) or detrusor-sphincter dyssynergia (DSD) after spinal cord injury (SCI) or bladder outlet obstruction in rats. Intrathecal, intravenous, or dietary glycine inhibits both bladder and urethral activity in normal and spinal cord injury (SCI) rats. Therefore, glycine might be a useful agent for the treatment of DO. Intrathecal muscimol and baclofen (GABA_A and GABA_B agonists, respectively) also inhibit non-voiding bladder contractions by suppressing C-fiber bladder afferents in SCI rats. They also improve DSD by suppressing Onuf's nucleus and C-fiber bladder afferents. Baclofen is approved for the treatment of DO in SCI patients, but this agent has not been widely used because the therapeutic window of the drug is modest and the dose is limited by side-effects. Glutamic acid decarboxylase (GAD), the GABA synthesis enzyme, gene delivery by using non-replicating herpes simplex virus (HSV) vectors inhibits DO by suppressing C-fiber bladder afferents without affecting voiding contraction in SCI rats. Therefore, GAD gene therapy can restore urine storage function without affecting voiding function; it would be more beneficial than drug therapy for the treatment of urinary problems in SCI patients.

Keywords

gamma-aminobutyric acid; gene therapy; glutamate; glutamic acid decarboxylase; glycine

1. INTRODUCTION

In the central nervous system, some amino acids are known to be important neurotransmitters.¹ For example, glutamate is a major excitatory neurotransmitter in the central nervous system, and facilitates the micturition reflex.² N-methyl-D-aspartate and alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid/kainate receptors are involved

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CONFLICT OF INTEREST

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in glutamatergic transmission in the micturition reflex pathway. Although glutamatergic receptor antagonists are theoretically useful for the treatment of overactive bladder because of glutamatergic neurotransmission involvement at various central nervous system sites, bladder-specific agents are not currently available. In contrast, glycine and gamma-aminobutyric acid (GABA) are major inhibitory neurotransmitters in the central nervous system, and they inhibit the micturition reflex at the level of the lumbosacral cord.^{3,4} Therefore, some approaches, including an advanced drug delivery system or gene therapy of glycine or GABA could contribute to the treatment of overactive bladder.

Micturition depends on the coordination between the bladder and external urethral sphincter.⁵ However, spinal cord injury (SCI) rostral to the lumbosacral level impairs voluntary and supraspinal control of voiding. In our previous study, the micturition reflex disappeared immediately after transection of the thoracic cord in rats, and the glycine level in the lumbosacral cord showed a transient increase.⁴ However, the micturition reflex reappears after 2–4 weeks and the glycine and glutamic acid decarboxylase (GAD), the GABA synthesis enzyme, levels of the lumbosacral cord decrease at this time point.^{4,6} SCI bladders exhibit detrusor overactivity (DO), and bladder-sphincter coordination is impaired, leading to detrusor-sphincter dyssynergia (DSD).⁷ Therefore, glycine and GABA are likely to be involved in the control mechanism of the micturition reflex after SCI. The present review summarizes recent advances in neurophysiology and neuropharmacology of glycine and GABA in respect to central nervous control of the micturition reflex.

2. EFFECTS OF GLYCINE ON BLADDER FUNCTION IN NORMAL AND SCI RATS

Spinal glycinergic mechanisms inhibit spinal afferent and efferent limbs of the spinobulbospinal and spinal micturition reflex pathways because intrathecal glycine inhibits the isovolumetric bladder contractions in normal and SCI rats.⁴ The parasympathetic preganglionic neurons innervating the bladder continuously receive both excitatory and inhibitory inputs during the collecting phase because intrathecal strychnine (a glycine receptor antagonist) elevates the baseline pressure of isovolumetric bladder contractions in normal rats. Intravenous⁸ or dietary (1–3%)⁹ glycine inhibits bladder activity in normal and SCI rats because glycine can cross the blood-brain barrier. Therefore, glycine may be a useful agent for the treatment of overactive bladder.

Electrical stimulation or injection of carbachol (a cholinomimetic agent) into the rostral pontine reticular formation (also known as the nucleus reticularis pontis oralis [PoO]) just ventrocentral to the pontine micturition center inhibits bladder contraction in cats and rats.^{10,11} The descending pathway from the PoO projects to lumbosacral inhibitory glycinergic neurons.¹¹

3. EFFECTS OF GLYCINE ON URETHRAL FUNCTION IN SCI RATS

Intrathecal glycine alters the urethral activity during bladder contraction from a pattern of DSD to detrusor-sphincter synergy in SCI rats.¹² Dietary glycine (1%) also improves DSD and decreases residual urine volume in SCI rats.¹²

4. EFFECTS OF GABA ON BLADDER FUNCTION IN NORMAL AND SCI RATS

GABA is synthesized from glutamate by GAD,¹ and is known to have an important role in the inhibitory regulation of micturition in spinal intact rats.³ Both muscimol and baclofen (GABA_A and GABA_B agonists, respectively) produce a dose-dependent inhibition of the C-

fiber-mediated non-voiding bladder contractions, and a decrease in micturition pressure in SCI rats.⁶ The effects of muscimol and baclofen are respectively antagonized by bicuculline and saclofen (GABA_A and GABA_B antagonists, respectively). Therefore, stimulation of spinal GABAergic mechanisms could be effective for the treatment of DO after SCI.

5. EFFECTS OF GABA ON URETHRAL FUNCTION IN NORMAL AND SCI RATS

After intrathecal application of muscimol or baclofen, urethral pressure shows urethral relaxation during isovolumetric bladder contractions in SCI rats.¹³ The effective dose to induce inhibition of urethral activity is lower compared with the dose that inhibits bladder contractions. In spinal intact rats, intrathecal application of bicuculline induces DSD-like changes.¹³ These results indicate that GABA_A and GABA_B receptor activation in the spinal cord exerts inhibitory effects on DSD after SCI. Decreased activation of GABA_A receptors due to hypofunction of GABAergic mechanisms in the spinal cord might be responsible in part for the development of DSD after SCI.

6. TIME COURSE OF CHANGES IN THE LEVEL OF GLYCINE OR GABA IN THE L6-S1 SPINAL CORD AFTER SCI OR BLADDER OUTLET OBSTRUCTION IN RATS

When the spinal cord is transected at the lower thoracic level, the lower extremities and bladder develop flaccid paralysis. The glycine level increases in the lumbosacral cord, and intrathecal strychnine induces bladder contraction.⁴ Therefore, elevated glycine levels might mediate acute spinal shock after SCI. In rats with chronic SCI, the bladder exhibits DO, and bladder-sphincter coordination is impaired, leading to DSD.⁷ At this time, glycine and GAD67 mRNA levels in the L6-S1 spinal cord decrease (54 and 55% reductions, respectively).^{4,6} Thus, hypofunction of the glycinergic or GABAergic inhibitory systems might be responsible for the development of DO and/or DSD after SCI.

In rats with chronic bladder outlet obstruction (2–4 weeks), a model of benign prostatic hyperplasia (BPH), the glycine level is decreased in the lumbosacral cord.¹⁴ The serum glycine level is also decreased in both patients with BPH and rats with chronic bladder outlet obstruction.^{14,15} Therefore, bladder activity and glycinergic neuronal activity in the lumbosacral cord may show an inverse correlation, and it may be possible to use serum glycine as an indicator of glycinergic neuronal activity in the spinal cord.

7. RELATIONSHIP AMONG GLUTAMATERGIC, GLYCINERGIC, AND GABAERGIC INTERNEURONS IN THE CENTRAL NERVOUS SYSTEM

Glutamatergic projections to the lumbosacral cord promote the micturition reflex and stimulate glycinergic neurons (Fig. 1).¹⁶ Glycinergic/GABAergic projections to the lumbosacral cord inhibit the micturition reflex and also inhibit glutamatergic neurons (Fig. 1).⁴ Intrathecal injection of strychnine or bicuculline does not completely restore reflex bladder contractions that are abolished by rectal distension in spinal intact rats, but simultaneous intrathecal injection of small doses of both drugs does so.¹⁷ Therefore, spinal inhibitory neurotransmitters, such as glycine and GABA, might have additive or synergistic inhibitory effects on bladder activity.

8. GAD GENE THERAPY ON DO IN SCI RATS

Baclofen is approved for treatment of DO in SCI patients,¹⁸ but this agent has not been widely used because the therapeutic window of this drug is modest and the dose is limited by side-effects. One potential approach in increasing GABA in the spinal cord with limited side-effects is the use of viral-mediated gene delivery (Fig. 2). Three weeks after replication-defective herpes simplex virus (HSV) vectors encoding genes of GAD (HSV-GAD) injection in SCI rats, the number and amplitude of non-voiding contractions are significantly decreased compared with sham and HSV-LacZ group (control vectors), but micturition pressure is not different among three groups.¹⁹ Because decreased non-voiding contractions and bladder contractility (i.e. a reduction in maximal voiding pressure) is assumed to reflect the suppression of afferent and efferent activity in the micturition reflex pathway, respectively,⁷ HSV-GAD treatment is likely to mainly inhibit the afferent limb of the micturition reflex. Desensitizing C-fiber afferents by systemic capsaicin administration suppresses non-voiding contractions without affecting the voiding reflex in chronic SCI rats²⁰; therefore, GAD gene therapy may initially inhibit DO by suppressing C-fiber bladder afferents without affecting voiding contraction. Thus, GAD gene therapy using non-replicating HSV vectors that can restore urine storage function without affecting voiding function would be more beneficial than drug therapy for the treatment of urinary problems in SCI patients.

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Fig. 1.

Relationship between glutamatergic and glycinergic/gamma-aminobutyric acid (GABA)ergic neurons and the micturition reflex pathway in the lumbosacral cord. Glutamatergic neurons (Glu) facilitate the afferent and efferent limbs of the micturition reflex, while glycinergic/GABAergic neurons (Gly/GABA) inhibit the afferent and efferent limbs of the micturition reflex and also inhibit glutamatergic neurons. These glutamatergic and glycinergic/GABAergic neurons receive input from the upper central nervous system as well as peripheral afferent neurons.

Fig. 2.

Methods of herpes simplex virus-glutamic acid decarboxylase (HSV-GAD) injections to the bladder wall in spinal cord injury rats. Injected HSV-GAD to the bladder wall infects the L6-S1 bladder afferents (i.e. dorsal root ganglia) and increases gamma-aminobutyric acid, and only inhibits bladder afferents. CNS, central nervous system; PMC, pontine micturition center.