

SUPPRESSION OF DETRUSOR OVERACTIVITY AND DETRUSOR-SPHINCTER DYSSYNERGIA BY GABA-RECEPTOR ACTIVATION AT THE LUMBOSACRAL SPINAL CORD IN SPINAL CORD INJURY RATS

Hypothesis / aims of study

Micturition depends on the coordination between the bladder and external urethral sphincter. However, in the chronic phase of spinal cord injury (SCI), the bladder exhibits detrusor overactivity (DO) and bladder-sphincter coordination is impaired, leading to detrusor-sphincter dyssynergia (DSD).¹ These lower urinary tract dysfunctions then produce various problems, such as urinary incontinence, recurrent urinary tract infection and vesicoureteral reflux with or without upper urinary tract deterioration. Gamma-aminobutyric acid (GABA) is known to be an important inhibitory transmitter in the central nervous system. Therefore, to clarify the role of GABAergic mechanisms in bladder and urethral function after SCI, we examined the effect of intrathecal application of GABA_A and GABA_B receptor agonists in SCI rats.

Study design, materials and methods

Adult female Sprague-Dawley rats were used 4 weeks after Th9-10 spinal cord transection.

(1) Continuous cystometry and external urethral sphincter electromyogram (EUS-EMG) were performed under an awake condition in order to examine the effect of intrathecal GABA_A and GABA_B agonists (0.001-1 µg; muscimol and baclofen, respectively) at the level of L6-S1 spinal cord. GABA_A and GABA_B antagonists (bicuculline and saclofen, respectively) were also used to confirm that the effect of agonists is mediated by activation of GABA receptors. (2) Urethral activity was also evaluated by simultaneous recordings of intravesical pressure under isovolumetric conditions and urethral perfusion pressure (UPP) under an awake condition before and after intrathecal muscimol or baclofen (0.001-1 µg). (3) Expression of glutamate decarboxylase 67 (GAD67), a GABA synthesis enzyme, mRNA in L6-S1 dorsal root ganglia (DRG), where bladder afferent nerves originate, and the lumbosacral spinal cord was assessed in spinal intact and SCI rats. Then the ratio of GAD67 to β-actin mRNA was compared between spinal intact and SCI rats.

Results

(1) Changes in bladder and EUS-EMG activity after intrathecal injection of muscimol or baclofen During awake cystometry, all spinalized rats showed nonvoiding bladder contractions (NVCs) before large amplitude voiding bladder contractions occurred. The number and amplitude of NVCs, and maximal voiding pressure (MVP) were 4.81 ± 0.49 , 28.6 ± 2.56 cm H₂O, and 53.6 ± 2.61 cm H₂O, respectively, before drug administration. Voiding efficiency was $62.3 \pm 5.2\%$. Both muscimol and baclofen (0.001-1 µg) produced a dose-dependent inhibition of the number (51-73% decrease) and amplitude (35-93% decrease) of NVCs, and a decrease in MVP. Dribbling overflow incontinence was noted after 1 µg of muscimol or baclofen in 50 % of SCI rats. The effects of muscimol and baclofen were antagonized by intrathecal bicuculline (0.01 µg) and saclofen (1 µg), respectively. Bursting activity of EUS-EMG was inhibited along with the inhibition of bladder activity by muscimol or baclofen. (2) Changes in simultaneous recordings of UPP and intravesical pressure During UPP measurements, DSD characterized by urethral pressure increases without pressure decreases during isovolumetric bladder contractions was observed in 88% of SCI rats. In these animals, the average increase in urethral pressure at the peak bladder contraction was 16.3 ± 2.62 cm H₂O above baseline urethral pressure. However, after intrathecal application of muscimol (0.1-1 µg) or baclofen (0.001-1 µg), UPP showed urethral relaxation (i.e., synergic pressure decrease) during isovolumetric bladder contractions. The effective dose to induce inhibition of urethral activity was lower (muscimol; 0.1 µg and baclofen; 0.001 µg) compared with the dose of muscimol and baclofen (1 µg) that inhibited bladder contractions. (3) Changes in the GAD67 mRNA level in DRG (L6 and S1) and the lumbosacral spinal cord after SCI The GAD67 mRNA/β-actin mRNA ratio (0.80 ± 0.18) in DRG of SCI rats was significantly lower (84% decrease) compared with spinal intact rats (5.01 ± 0.48). The GAD67 mRNA/β-actin mRNA ratio (0.39 ± 0.12) in lumbosacral cord of SCI rats was also significantly lower (55% decrease) compared with spinal intact rats (0.86 ± 0.02).

Interpretation of results

Intrathecal application of muscimol or baclofen inhibited the number and amplitude of NVCs, and MVP dose-dependently, and induced urinary retention with dribbling incontinence at the highest dose in SCI rats. These effects were mediated by GABA_A and GABA_B receptors, since they were completely inhibited by selective antagonists, bicuculline and saclofen, respectively. Urethral pressure rise during isovolumetric bladder contractions was inhibited and changed to the synergic pattern by intrathecal muscimol or baclofen at the dose lower than that inhibiting bladder activity, suggesting that intrathecal application of either muscimol or baclofen inhibits both the bladder and urethral activity simultaneously with higher sensitivity for urethral sphincter activity. The current study also shows that GAD67 mRNA level in the L6-S1 DRG and the lumbosacral spinal cord was decreased by 84% and 55%, respectively after SCI, suggesting that hypofunction of the GABAergic inhibitory system is involved in the development of DO and DSD after SCI.

Concluding message

This study indicated that both GABA_A and GABA_B receptor activation in the spinal cord exerts the inhibitory effects on bladder/urethral hyperactivity induced by SCI with higher sensitivity for urethral sphincter activity. Desensitizing C-fiber afferents by systemic capsaicin administration suppresses NVCs and DSD.^{2,3} Interestingly, these findings are similar

to those after GABA receptor activation in the present study, suggesting that GABA receptor activation inhibits DO and DSD via inhibition of C-fiber bladder afferents. Because hypofunction of GABAergic inhibitory system seems to be responsible at least in part for the development of DO and DSD after SCI, therefore, stimulation of spinal GABAergic mechanisms could be effective for the treatment of DO and DSD in patients with SCI.

References

1. Prog Brain Res (2006) 152:59-84.
2. Brain Res (1995) 678: 40-48.
3. J Urol (2004)171: 478-482.

FUNDING: Source of Funding: NIH DK57267, DK68557 and P01 DK44935

ANIMAL SUBJECTS: This study followed the guidelines for care and use of laboratory animals and was approved by University of Pittsburgh Institutional Animal Care and Use Committee