423

Liao L[1], Zhang F[1]
1. *Dept of Urology, China Rehabilitation Research Centre, Capital Medical University, Beijing, China*

INVESTIGATION ON THE ROLE OF NEUROCHEMICAL PLASTICITY OF NOS ISOMER IN THE MICTURITION REFLEX AFTER SPINAL CORD INJURED RATS

Hypothesis / aims of study

Nitric oxide (NO), a neurotransmitter in autonomic reflex pathways, plays an important role in the neural pathways of the lower urinary tract. There is also considerable evidence to suggest that neuronal nitric oxide synthase (nNOS), the enzyme system responsible for NO synthesis, is plastic and could be upregulated in the peripheral, spinal and supraspinal segments of the micturition reflex following diseases such as cystitis, bladder overactivity developed by cerebral infarction or bladder outlet obstruction. These observations suggest that NO might play a role in the development and maintaining of bladder overactivity. It has also been reported that overactive bladder (OAB) caused by noxious chemical irritation was partially reversed by intrathecal administration of n-NOS inhibitor, although, whether the same effect can be achieved so as to alleviate the detrusor overactivity on spinal cord injured rats is still unknown. The aim of this study was to evaluate the differential distribution of nNOS\iNOS-immunoreactivity (IR) in bladder afferent neurons associated segmental level of spinal cord intact rats and rats following spinal cord injury (SCI) among different period,and also to investigate the effects of intrathecal administration of different NOS inhibitor on detrusor overactivity with rats after 1 month SCI.

Study design, materials and methods

A total of 40 adult female Sprague-Dawley rats weighing 200 to 250 g were used in this study. SCI was induced by Th 9-10 spinal cord transection after laminectomy. Intact rats (n=7) and rats at 4 weeks (n=9) following the SCI , under urethane (1.2g/kg) anaesthesia ,the bladder function was evaluated by continuous cystometry to examine the effect of intrathecal application of different NOS inhibitor . A vein injection pinhead was inserted via a midline abdominal incision into the bladder through the bladder dome, and connected with an infusion pump. The baldder was filling with physiological saline of room temperature at a rate of 0.08 mL/min to elicit bladder contraction. Cystometric parameters such as mean amplitudes (MA) and number of non-voiding contractions (NVCs), Maximum bladder volume (MBV) and maximum voiding pressure (MVP) were evaluated before and after intrathecal administration of vehicle (physiological saline) and different NOS inhibitor (Spermidine Trihydrochloride : nNOS inhibitor and S - Methylisothiourea Sulfate/SMT: iNOS inhibitor) , respectively, at the level of the L6-S1 spinal cord. In 24 rats (6 per group) of intact, 1, 4 and 8 weeks following SCI, the distribution of nNOS\iNOS (IR) was also accessed at the level of L6 and S1 spinal cord segments.

Results

In intact rats, the cystometric parameters including MA, NVCs, MBV and MVP were all stable before the drug administration. There were not any significant changes on those cystometric parameters after intrathecal injection of either nNOS inhibitor or iNOS inhibitor. In the SCI rats, MBV measured after intrathecal injection significantly increased from 1.06±0.70 mL to 1.58±0.94 mL at 1 μmol of nNOS inhibitor, but there were not significant changes on MVP, MA and NVCs. Intrathecal injection of iNOS inhibitor (at 1 μmol.) did not significantly change any results from CMG.

Compared with the intact rats, a significant increase in the number of nNOS IR was detected in the L6-S1 spinal segments both among the lateral edge of dorsal horn (DH) at lamina I and ventral horn motoneurons (VH) following 4 weeks SCI rats: DH from 2±1.5 to 10.3±7.9 nNOS IR cell profile/section, VH from 4.3±1.5 to 9.7±4.4 nNOS IR cell profile/section. There were no significant change after 1 week of SCI (DH: 5.5±2.7 nNOS IR cell profile/section, VH: 4.9±2.0 n NOS IR cell profile/section) or after 8 weeks of SCI (5.3±3.2 nNOS IR cell profile/section, 6.7±1.5 n NOS IR cell profile/section). There were no significant changes between 4 weeks and 8 weeks after SCI. The expression of iNOS was not detected both intact group and SCI one.

Interpretation of results

In this study, there were no significant differences in the urodynamic parameters after intrathecal injection of NOS inhibitor in spinal intact rats. These results indicate that the spinal NO contained in pathways does not play a role in the normal micturition reflex. In the SCI rats, only the maximum baldder volume were transiently increased after intrathecal injection of nNOS inhibitor, indicate that, after SCI, NO may facilitate transmission at an interneuronal synapse on the ascending limb of the reflex pathway; this could enhance excitatory input to the lumbar-sacral spinal micturition center and reduce the volume threshold for inducing micturition without altering the intensity of pregangbonic efferent firing or the amplitude of the bladder contractions. The significant increase of nNOS IR in the L6 spinal segments lateral edge of dorsal horn in SCI rats 4 weeks indicated that the nNOS IR in bladder afferent neurons associated segmental level of spinal cord is plastic and can be up-regulated by the chronic SCI. During both the spinal shock period and chronic SCI period, the nNOS IR showed a mild increase. The upregulated n-NOS IR in ventral horn motoneurons could have been induced by the afferents from the overdistended bladder in the animal model with detrusor-sphincter dyssynergia (DSD) after SCI.

Concluding message

The results obtained from this study indicate that: (1) spinal NO does not play a role in the central reflex pathway or lumbar-sacral spinal micturition primary center during normal micturition. (2) the changes in the neurochemical porperties of thoese neurons after SCI may be mediated by pathological changes in the target organ and subsequently affect the function of those organ. (3) the intervention for these changes may find a new solution for treatment for the dysfunction, e.g. neurogenic bladder after SCI.

Specify source of funding or grant	**National Technology R&G Program**
Is this a clinical trial?	**No**
What were the subjects in the study?	**ANIMAL**
Were guidelines for care and use of laboratory animals followed or ethical committee approval obtained?	**Yes**
Name of ethics committee	**Ethics committee of China Rehabilitation Research Center**