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Sensory Neuronal Change after Intravesical Electrical Stimulation in Spinailized Rat

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The clinical benefits of intravesical electrical stimulation (IVES) in patients with increased residual urine or reduced bladder capacity have been reported. However, studies on the underlying mechanism of IVES has been limited to the Aδ afferent and parasympathetic neurons. This study investigated the changes in the calcitonin gene-related peptide (CGRP), substance P (SP), and nitric oxide synthase (NOS) expression in the thoracolumbar and lumbosacral dorsal root ganglia (DRG) of spinalized rats to determine the effect of IVES on the C fiber afferent nerve. Forty Sprague-Dawley rats were divided into normal controls (n=10); IVES treated normal rats (n=10), spinalized rats (n=10), and IVES treated spinalized rats (n=10). IVES was performed for 2 weeks (5 days a week). IVES was started 3 weeks after spinalization in the spinalized animals. All animals had the DRG removed at the thoracolumbar (T13-L2) and lumbosacral (L5-S1) level. Changes in the CGRP, SP and n-NOS levels at the DRG were measured by western-blot analysis. The relative density of the CGRP and SP following spinalization was significantly higher compared to the controls in both the T13-L2 and L5-S1 DRG. However, IVES in the spinalized rat significantly decreased the relative density of the CGRP and SP compared to the rats with spinalization alone. A significant increase in the relative density of n-NOS was detected in the L5-S1 DRG following spinalization. However, the density of n-NOS was significantly lower after IVES in both the T13-L2 and L5-S1 DRGs. In conclusion, IVES significantly reduced the CGRP, SP and n-NOS levels in the DRG of spinalized rats. CGRP, SP and n-NOS are the main factors that contribute to the hyperexcitability of the micturition reflex after spinal cord injury. These results suggest that the bladder C fiber afferent is also involved in modulating the micturition reflex by IVES.

Key Words: Electric stimulation, spinal cord injury, afferent C-fibres, dorsal root ganglia

INTRODUCTION

Kotona originally introduced intravesical bladder stimulation (IVES) in 1959.[1] Since 1984, IVES has been used in the United States as a rehabilitative technique for children with myelodysplasia.[2] Worldwide, several other investigators have used IVES to treat neurogenic bladder dysfunction secondary to a variety of factors, including spinal cord injury (SCI), myelodysplasia and other neurological diseases.[3,4] Impressive results from IVES have been reported, including the restoration of bladder sensation with filling and stimulation of the detrusor contraction, conscious urinary control and a significant increase in bladder capacity. These benefits have been achieved without harmful effects on the upper urinary tract. While this goal was indeed achievable in some patients, it has been recently recognized that the majority are unable to have complete conscious control of the lower urinary tract after undergoing IVES.[5] Consequently, the initial goals of IVES have been changed to just improving bladder compliance and sensation.

In experimental studies to obtain objective evidence of the initial goals, it was shown that IVES involves the direct activation of the Aδ afferents from the low-threshold bladder mechanoreceptors; the sensory system responsible for initiating and maintaining the micturition reflex.[6]

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For a better theoretical understanding of the working mechanism of IVES, this study aimed to measure the effect of IVES on the change in the neuropeptides of the C afferent fiber (CGRP, SP) and nitric oxide synthase (NOS), which has been implicated in the pathogenesis of bladder hyperreflexia following spinal cord injury.

MATERIALS AND METHODS

Animals and surgical procedure

Forty Sprague-Dawley rats were divided into normal controls (n=10); IVES treated normal rats (n=10), spinalized rats (n=10), and IVES treated spinalized rats (n=10). IVES was started 3 weeks after spinalization in the spinalized animals.

The spinal cord was transected at the T9-T10 under general anesthesia. The space between the retracted ends of the spinal cord was packed with Gelfoam and the incision was sutured. An antibiotic (150 mg/kg, ampicillin, s.c.) was administered 1 day prior to surgery and for 7 days postoperatively. The bladder was evacuated twice daily by manual expression.

For the IVES experimental procedure, the abdomen opened through via a midline incision, A polyethylene catheter (PE-50) was implanted into the bladder through the dome. After a platinum wire was inserted into catheter, the catheter was tunneled subcutaneously and an orifice was made on the back of the animal. The intravesical electrode was used as the cathode, a brass plate under the abdominal skin at the level of the bladder served as the anode. IVES was administered with a constant current stimulator for 2 weeks (5 days a week). Continuous stimulation for 10 minutes was used with a frequency of 20 Hz and the intensity of up to 10 mA.

Western blotting

All animals had the DRG removed at the thoracolumbar (T13-L2) and lumbosacral (L5-S1) level. The DRGs were immediately immersed in liquid nitrogen. The DRG was homogenized in a 50 mM Tris buffer. The homogenate was then centrifuged at 13,000g for 15 min at 4℃. The supernatant was decanted from the pellet and used for all western blot analyses. The protein concentration in the homogenate was measured using the BCA kit (Pierce, Rockford, IL, U.S.A.). Equal amounts of protein (60 ug) were size fractionated by 12% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred on to a PVDF membrane (Bio-Rad, Hercules, CA, U.S.A.). The blots were placed in blocking buffer for 1 h at room temperature and then incubated with the primary polyclonal antibodies to CGRP (Santa cruz, San Francisco, CA, U.S.A.), Substance P (SP, Oncogene, Cambridge, MA, U.S.A.) and n-NOS (BD transduction, Flanklin lakes, NJ, U.S.A.) for 1 h at room temperature. The blots were washed three times for 30 min in with washing buffer and incubated with horseradish peroxidase conjugated with IgG (Santa Cruz, San Francisco, CA, U.S.A.) diluted in 2.5% (w/v) non-fat milk in a washing buffer. The membranes were subsequently washed with buffer three times for 30 min and enhanced with a chemiluminesence reagent (ECL kit, Amersham, Arlington Height, IL U.S.A.). The blots were then exposed to autoradiographic film (Kodak, Rochester, NY, U.S.A.) and the intensity of the specific immunoreactive bands was quantified using densitometric scanning analyses. The densitometric units of the specific bands are expressed relative to the values of the background units.

Statistics

The data is presented as the relative density of the immunoblots from the DRG and is expressed as a mean (SEM). The comparison between the different groups was done using a univariate analysis of variance (ANOVA). A p value < 0.05 was considered statistically significant.

RESULTS

Thoracolumbar DRG (T13-L2)

The relative density of CGRP (4239.9 ± 318.4), SP (7662.1 ± 558.2) following spinalization was higher ($^*p < 0.05$) (Fig. 1). However, no significant change was detected in the relative density of

n-NOS (5097.9 ± 465.2) compared to the control animals (4242.1 ± 197.6) in T13-L2 DRGs. IVES in normal rats caused a significant increase in the CGRP (5636.9 ± 453.6) and SP (13972.1 ± 1994.5) levels compared to the controls (22.3 ± 1.4, 4431.1 ± 375.7) ($^{\dagger}p<0.05$), while the relative n-NOS density (3897.8 ± 245.2) was unaffected. The relative density of all neuropeptides (CGRP, 1425.7 ± 176.5, $^{\S}p<0.05$; SP, 4186.6 ± 367.3, $^{\S}p<0.05$: n-NOS, 1760.3 ± 245.8, $^{\S}p<0.05$) in the spinalized rats that underwent IVES was significantly lower than the rats with spinalization alone (Fig. 2).

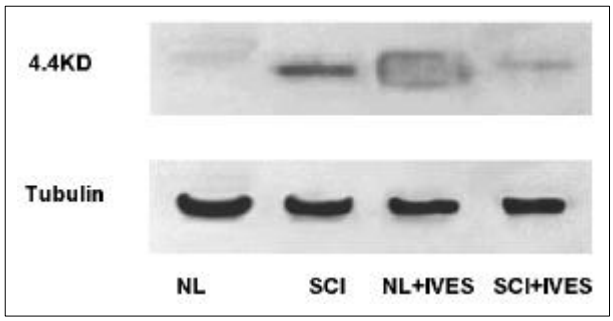

Fig. 1. Immunoblots of the dorsal root ganglia (T13-L2) using CGRP antibodies.

Lumbosacral DRGs (L5-S1)

The relative density of CGRP (7623.1 ± 645.3) and SP (18502.3 ± 2123.5) was significantly higher after spinalization ($^{*}p<0.05$), which was like to the thoracolumbar DRGs. In contrast, the relative density of n-NOS (3999.7 ± 255.5) was significantly higher in the lumbosacral DRGs ($^{*}p<0.05$). In contrast to the thoracolumbar DRGs, IVES in the normal rats did not alter the relative density of CGRP (3591.5 ± 255.7) and SP (7545.7 ± 688.4) in the lumbosacral DRGs. Like the thoracolumbar DRGs, the relative density of n-NOS was unchanged in the IVES treated normal rats compared to the controls. The relative density of all neuropeptides (CGRP, 1042.5 ± 132.5, $^{\S}p<0.05$; SP, 3725.1 ± 288.5, $^{\S}p<0.05$: n-NOS, 2077.1 ± 188.4, $^{\S}p<0.05$) in the spinalized rats that underwent IVES was significantly lower when compared to the rats with spinalization alone. This result was similar to that observed with the thoracolumbar DRGs (Fig. 3).

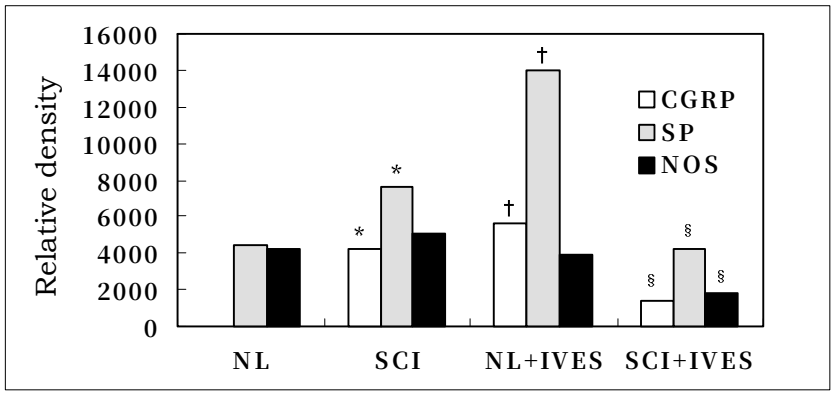

Fig. 2. Proportion of the relative densities of CGRP, SP and NOS in the DRG (T13-L2). The relative density of CGRP and SP following spinalization (SCI) was significantly higher compared to the control (NL). In addition, IVES in the normal rats (NL + IVES) caused a significant increase in the CGRP and SP levels. IVES in the spinalized rats (SCI + IVES) significantly decreased the relative densities of CGRP, SP and NOS. (*Nl vs SCI, †NL vs NL + IVES, §SCI vs SCI + IVES).

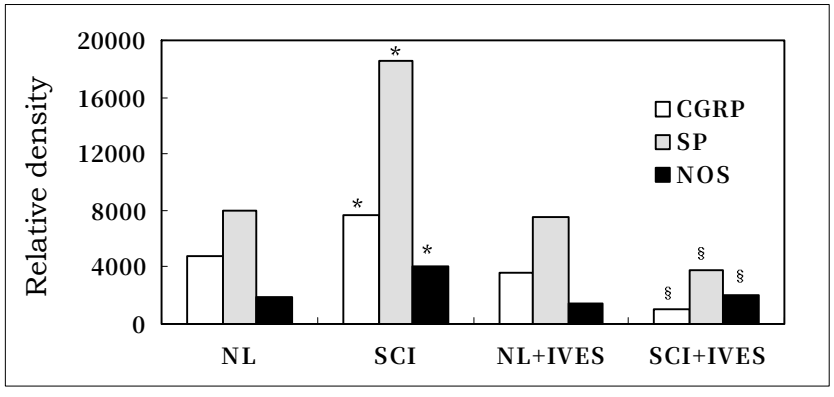

Fig. 3. Proportion of the relative densities of CGRP, SP and NOS in the DRG (L5 -S1). The relative density of CGRP, SP and NOS following spinalization (SCI) was significantly higher than the control (NL). Like the thoracolumbar DRGs, IVES in the spinalized rat (SCI + IVES) significantly decreased the relative densities of CGRP, SP and NOS compared to the rats with spinalization alone (SCI).

DISCUSSION

Several studies have investigated the working mechanism of IVES.[6-10] In the experimental studies, it was shown that IVES involves the direct artificial activation of the Aδ afferents from the low-threshold bladder mechanoreceptors, the sensory system responsible for both initiating and maintaining the micturition reflex.[6] Recently, it was found that the IVES-induced modulation of the micturition reflex is due to an enhanced excitatory synaptic transmission in the central micturition reflex pathway.[9] However, previous experimental studies were limited to the myelinated Aδ afferents in healthy rats. There is evidence that the C afferent fibres are involved in many pathological condition involving the lower urinary tract, including urinary bladder hyperreflexia.[11] Moreover, it has been recently recognized that the majority of patients with a pathologic bladder are unable to achieve complete conscious control of the lower urinary tract after undergoing IVES. Therefore, a better theoretical understanding of the working mechanism of IVES is necessary for improving the treatment success rate.

This study showed that IVES significantly decreased the CGRP, SP and n-NOS levels in the DRG from the spinalized rats, which are the main factors contributing to the hyper-excitability of the micturition reflex after a spinal cord injury. It is clear from previous studies that spinal cord injury induces a functional and morphological plasticity in the bladder afferent pathway. The micturition reflex in chronically spinalized animals is characterized by bladder hyperactivity and simultaneous contractions of the bladder and external urethral sphincter (detrusor-sphincter-dyssynergia), which leads to large residual urine volumes and detrusor muscle hypertrophy.[12] It has been shown that in chronic SCI animals, the reflex micturition and bladder instability is mediated by the C-fibre afferents.[13,14] CGRP and SP are used to mark the activity of the C-afferent fibres.[15] Therefore, the increased number of bladder afferents expressing CGRP and SP is one of main factors that contribute to the hyper-excitalbility of the micturition reflexes. It is known that the lumbosacral DRG content of the CGRP and SP increases after a spinal transection[16] and cystitis.[17] In this study the relative density of CGRP and SP in the lumbosacral DRGs following spinalization was also significantly higher compared to the control. However, IVES in the spinalized rats significantly decreased the relative density of CGRP and SP compared to the rats with spinalization alone. Consequently, it is proposed that IVES results in an inhibition of the pathological reflex conveyed by the C-afferent fibres.

Furthermore, both the relative CGRP and SP densities following spinalization were also significantly higher compared to the control in the thoracolumbar DRGs. In addition, IVES in the spinalized rats decreased the relative density of these neuropeptides compared to rats with spinalization only. The thoracolumbar DRGs are the origin of the sympathetic (inhibitory) innervation of the bladder. Thus, these changes in the thoracolumbar DRGs might be indicative of the simultaneous activation of an inhibitory mechanism that counteracts the development of bladder hyperactivity.

The possibility that neuronal NOS participates in the pathophysiology of SCI has recently been investigated. Vizzard[18] reported that chronic SCI increased NOS-ir in the lumbosacral (L6, S1) and rostral lumbar (L1, L2) dorsal root afferent neurons and this increase was most striking in the L6-S1 afferent neurons. In contrast, inflammation of the bladder induced a significant up-regulation of NOS-ir in the bladder afferents projecting to the rostral lumbar spinal cord (L1, L2), while the NOS level in the lumbosacral DRGs (L6, S1) was unaffected.[17] The function of the NO formed enhanced NOS expression in the bladder afferent following spinalization or inflammation is uncertain. However, although NO does not appear to be involved in the normal micturition reflex in rats, NO does appear to play a role in facilitating of the micturition reflex in a pathologic bladder in a similar way as SCI. In this study, a significant increase in the relative n-NOS density was detected in the L5-S1 DRG following spinalization. However, the n-NOS density was reduced significantly after IVES in both the T13 - L2 and L5 - S1 DRGs. Therefore, it is apparent that the inhibitory effect of IVES on the hyperactive bladder after SCI occurs via a reduction in the n-NOS level in the bladder afferent neurons.

The remaining question is how does IVES

causes a decrease in concentration of neuropeptides that contribute to the hyperexcitability of the micturition reflex after a spinal cord injury. Urinary bladder neuropeptide quantification after IVES may provide an answer. In our unpublished observations, IVES in spinalized rats reduced both the relative densities of CGRP and SP in the bladder compared to rats with spinalization only. This is due to the fact that both CGRP and SP are produced only in the neuronal bodies in the dorsal root ganglia and are transported to the bladder,[19] Capsaicin sensitive primary afferents desensitization in the bladder by IVES causes a decrease in the CGRP and SP levels in the DRGs.

There were different results of IVES in the normal rats than in the spinalized rats. IVES in normal rats did not alter the either of the relative CGRP and SP densities in the lumbosacral DRGs. The micturition reflex in normal rats is mediated by the Aδ fibers in contrast to the spinalized animals where these fibers have only modulatory effects on the reflex mediated by the C-fibers. In normal rats, the stimulation intensity used in the IVES is well below the threshold for electrical activation of the C afferents because the C-fiber bladder afferent in normal rats are less excitable. However, IVES in normal rats caused a significant increase in the CGRP and SP levels in the thoracolumbar DRGs compared to the controls. It is possible that this is an inhibitory effect secondary to sensitization after an IVES-induced bladder contraction in normal rats.

In conclusion, IVES modulates the micturition reflex conveyed by the C-afferent fibers. However, to determine the IVES mechanism on a C fiber precisely, quantitative measurements of the neuropeptides at the dorsal root ganglia and bladder in the same animal should be made and the mRNA coding for them should be determined.

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