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Early capsaicin intervention for neurogenic bladder in a rat model of spinal cord injury

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ABSTRACT

We explored capsaicin pretreatment, prior to spinal trauma, as a method to prevent the development of neurogenic detrusor overactivity (NDO) and urethral-bladder dyssynergia reflex after spinal cord injury (SCI). In addition, the duration of effect of capsaicin therapy on NDO in a rat model of SCI was investigated. Two sets of experiments were performed on female Sprague Dawley rats transected at the T9–T10 spinal level. First, SCI rats received capsaicin (125 mg/kg s.q.) 3–4 days before and 4–5 days after SCI. Cystometrograms (CMG) was performed 4 weeks after injury. In the second set of experiments, serial CMG in the same SCI animal was performed after one time injection of capsaicin (125 mg/kg s.q.) 4 weeks after spinalization. There were no differences in intercontraction intervals, voiding efficiency, or voiding pressure between the capsaicin pretreated and control SCI rats. However, the number of uninhibited detrusor contractions decreased 4 weeks after injury. We found that a single dose of capsaicin suppressed uninhibited detrusor contractions for 34 days in the chronic SCI animals. Early therapy with capsaicin was able to prevent/reduce detrusor hyperreflexia in spinal cord injured animals 4 weeks after injury. Early vanilloid therapy may prevent development of urologic sequelae after SCI.

One of the most important priorities of all spinal cord injury (SCI) clinical and basic research is immediate and early therapy to prevent the sequelae of SCI (3). Although there has been significant progress in early systemic treatment, such as anti-inflammatory steroid administration to minimize the size and severity of spinal cord damage, there has never been an investigation of early urologic intervention to prevent and minimize life-long urologic complications (11). It would be ideal if early intervention into the bladder at the acute SCI phase can prevent the future appearance of such sequelae as neurogenic detrusor overactivity (NDO) and bladder induced autonomic dysreflexia.

A number of investigators and our laboratory have observed that intravesical vanilloid treatment can eliminate uninhibited bladder contractions in chronic SCI rats (2, 4). In this regard, it may be possible to prevent even the first appearance of NDO by giving capsaicin at the spinal shock phase. However, it is not known whether this type of prophylactic capsaicin (vanilloid) therapy would have the same effect on NDO as when given after chronic SCI. In addition, despite a large number of studies that have been done in urologic research with vanilloids such as capsaicin and resiniferatoxin, the duration of effect of these drugs on suppression of uninhibited bladder contractions in a SCI model has yet to be determined. The purpose of this experi-

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ment was to study whether early capsaicin treatment, given before spinal transection, can reduce or prevent the appearance of detrusor overactivity in SCI rats and determine the duration of effect of a single dose of capsaicin injection.

MATERIALS AND METHODS

Rat spinal cord injury. Female Srague Dawley rats (250–300 g) were anesthetized with halothane and a T9–T10 spinal laminectomy was performed. The underlying dura and spinal cord was transected with a number 11 scalpel blade, and the bone was scraped with a right-angled sharp dental pick to ensure complete transection. The resultant space between cut ends of the cord was filled with Gelfoam. The muscle layer and overlying skin were closed with suture and the animals were treated with antibiotic (ampicillin 100 mg/kg, s.q., every day beginning with the day of surgery for 7 days). The bladders of the spinalized animals were expressed two times daily at approximately 12-hour intervals by gentle pressure and perigenital stimulation for the period of the study.

Capsaicin injection. Capsaicin was dissolved in vehicle (10% ethanol, 10% Tween 80, 80% saline) to a concentration of 10 mg/mL. In the first set of studies, SCI animals (n = 5) received (125 mg/kg s.q.) of capsaicin 3–4 days before SCI and capsaicin booster shots (125 mg/kg) 4–5 days after the surgery. The injection was divided and 50, 25, and 50 mg/kg and was given subcutaneously every 12 hours in that order. Spinal cord injured animals without any treatment were used as control (n = 6). Cystometrograms (CMGs) were performed in all animals 4 weeks after injury because detrusor overactivity in SCI rats is usually established in 3–4 weeks after the injury (4, 9, 10).

In the second series of experiments, serial CMGs in another 6 SCI animals were performed after one time injection of capsaicin (125 mg/kg s.q.) 4 weeks after spinalization (4, 9). CMG was performed before and every 4–6 days after the injection.

Transvesical cystometrogram. Cystometrogram (CMG) was performed in awake/restrained animals. Under 0.4% halothane anesthesia, a midline abdominal incision was made and a PE50 catheter with fire-flared ends was passed through the bladder dome to access the bladder. The catheter tip was secured via ligation at the bladder entrance. The abdominal wall and overlying skin was separately closed with suture. Following surgery, the animals were immediately positioned and secured in a Ballman-type restraining device. The bladder catheter was connected to a Statham pressure transducers via a 3-way stopcock. The bladder infusion line connected to a saline filled syringe and mounted upon a Harvard Micron pump was also connected to the 3-way stopcock. The first two hours of CMG were considered as the recovery and acclimatization period. Thereafter, the voiding efficiency (VE), intercontraction interval (ICI), voiding pressure (VP), and number of uninhibited contractions (UIC) were evaluated.

A PE90 catheter was placed inside the bladder as above and the other end was brought out below the dorsal neck area via a subcutaneous tunnel in the 3 spinalized rats undergoing serial CMGs after one time capsaicin injection. The catheter was sealed with a flare until each CMG.

Electromyogram. Two animals from the control group also underwent external urethral sphincter electromyogram evaluation during CMG. Fine wire electrodes (50 μm diameter; M. T. Giken, Tokyo, Japan) were placed percutaneously into the pelvic floor and external urethral sphincter. The raw data from the electromyogram was fed through a window discriminator.

Data analysis. To evaluate the statistical significance the Student t test was used. The statistical analysis was computed with the GraphPad Prism program. Values are presented as means ± S.E. $P < 0.05$ was considered significant.

RESULTS

All control for spinal cord injured rats showed uninhibited contractions (Fig. 1A). The number of uninhibited contractions was markedly decreased in the early capsaicin pretreatment group (8.0 ± 0.6 UIC/void) compared to control (3.3 ± 0.6 UIC/void) (Fig. 3D, $p < 0.001$). Some animals displayed total suppression of UIC (Fig. 1B). All animals that underwent electromyography showed increase in external urethral sphincter activity at both uninhibited and voiding bladder contractions (Fig. 2). There were no differences in VE (Fig. 3A, $p = 0.74$), ICI (Fig. 3B, $p = 0.42$), or VP (Fig. 3C, $p = 0.31$) between the capsaicin pretreated and control SCI rats. The VE, ICI, and VP in the pretreated and control SCI rats were 62.7 ± 4.5 and 65.0 ± 3.5%, 574.5 ± 60.2 and 515.9 ± 37.4 seconds, and 53.1 ± 1.6 and

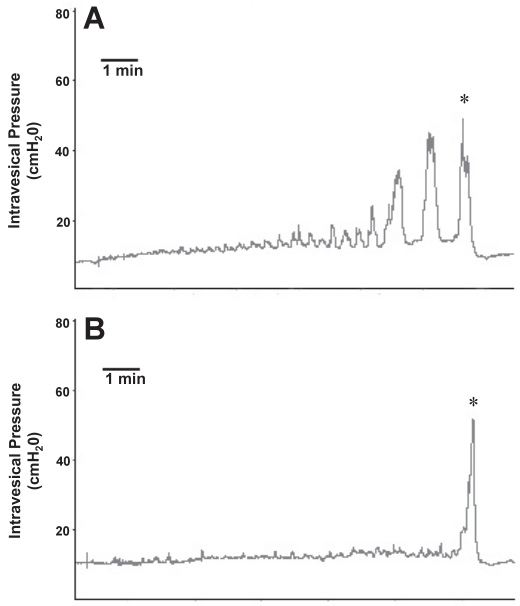

Fig. 1 Cystometrograms (CMGs) of spinal cord injured rats 4 weeks after the injury without (A) and with early capsaicin pretreatment (B). *: voiding contraction

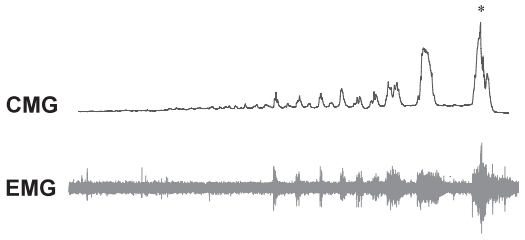

Fig. 2 Cystometrogram (CMG) and simultaneous electromyogram (EMG) of the external urethral sphincter show increased EMG activity at all bladder contractions in control animals. *: voiding contraction

48.8 ± 4.6 cmH_2O, respectively.

Of the six animals that underwent serial cystometrograms (CMGs) following subcutaneous injection of capsaicin 4 weeks after SCI, 3 died within 1–2 week after the first CMG. Of the remaining 3 animals only 1 showed no uninhibited contractions, this was observed 14 days post capsaicin injection. However, there was subsequent severe urinary tract infection and was not used for further evaulation. The remaining 2 rats were able to undergo CMGs 28 and 34 days after capsaicin injection, respectively. There were no apparent uninhibited contractions seen at both time points (Fig. 4), indicating that C-fiber desensitization can last at least 4–5 weeks after capsaicin treatment.

DISCUSSION

It is known that the micturition reflex among normal and chronic spinal injured animals are markedly different. A significant functional reorganization of the bladder-spinal cord-bladder reflex pathway occurs following SCI, with normally "silent" C-fibers becoming tonically active (3, 10). Direct anatomical evidence for this assertion is found in changes, that occur in the afferent system at both the level of the dorsal root ganglion (enlargement of afferent neuronal somata) (7) and the spinal cord afferent terminal fields (1, 6). This change in reorganization after SCI then leads to neurogenic detrusor overactivity and subsequent uninhibited bladder contractions as seen in our group of rats (Fig. 1A).

We observed an increase in external urethral sphincter eletromyographic activity at both uninhibited and voiding contractions (Fig. 2). The uninhibited bladder contractions observed in our spinalized rats did not show leakage of urine. This was probably due to the difference in the rat voiding mechanism compared to humans. Unlike humans, the rat requires high frequency oscillation of the external urethral sphincter in bursts for efficient voiding. The external urethral sphincter in the spinalized rat exhibits a tonic activity during the C-fiber mediated uninhibited contractions that are detrimental to voiding efficiency (7). However, high frequency oscillations in a bursting pattern were seen with the Aδ mediated voiding contractions (unpublished data) in these animals. Therefore, the rat may need a higher intravesical pressure than at the Aδ mediated voiding bladder contraction to overcome the deficiency in the assistance by the external urethral sphincter and show urinary leakage as in humans. It may also explain the no change in voiding efficiency despite our previous data (9) showing an improvement in detrusor-sphincter-dyssynergia in spinalized rats after capsaicin injection.

The development of NDO after SCI can be debilitating resulting in conditions from urinary incontinence to upper urinary tract damage. Incontinence not only impacts quality of life but it can be a source of medical problem related to skin turgor in spinal cord injured patients. It can also be a costly problem since the cost for products such as disposable pads and diapers are expensive. Moreover, NDO combined with detrusor-sphincter dyssynergia, commonly seen in these patients, can lead to damage of the upper urinary tract. The results from our study confirm that prophylactic capsaicin injection can reduce the number of nonvoiding contractions

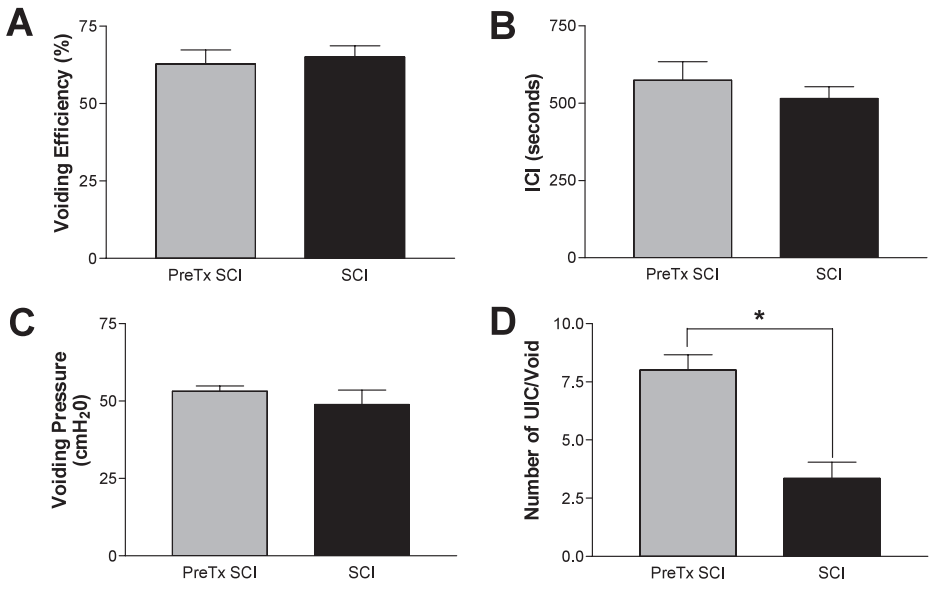

Fig. 3 (A) voiding efficiency, (B) intercontraction interval (ICI), (C) voiding pressure, and (D) number of uninhibited contractions (UIC) per void in early capsaicin pretreated (PreTx SCI) and control spinal cord injured (SCI) animals. *: $p < 0.01$

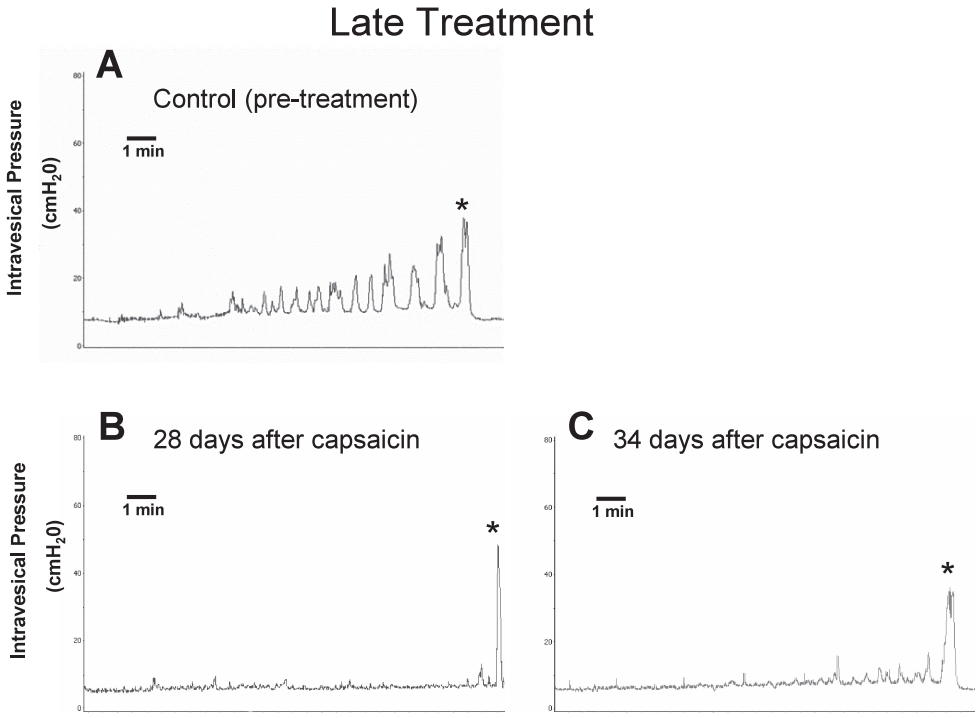

Fig. 4 Consecutive cystometrograms (CMGs) of a spinal cord injured rat. (A) 4 weeks after the injury (Control: pre-treatment). (B) 28 days after late capsaicin treatment performed 4 weeks after the injury. (C) 34 days after late capsaicin treatment performed 4 weeks after the injury. *: voiding contraction

seen with NDO in spinal cord injured rats. In addition, it provides proof of concept that prophylactic administration of the drug immediately after SCI can prevent the onset of NDO after the spinal shock phase. There has been no previous report examining the effects of early capsaicin administration on NDO in awake, spinalized rats as far as we know.

An ideal drug for the treatment of incontinence

would act selectively on the urinary bladder to suppress involuntary voiding without increasing the amplitude of bladder contractions or decreasing the efficiency of bladder emptying. We observed no differences in voiding efficiency, intercontraction intervals, or voiding pressure between the capsaicin pretreated and control SCI rats (Fig. 3A, B, C). However, the number of uninhibited bladder contractions was significantly reduced in the capsaicin pretreated group (Fig. 3D). This indicates that prophylactic blockage of C-fiber afferents before the emergence of C-fiber hyperexcitability could be beneficial in a clinical setting of patients with acute SCI. The benefits of this early blockade of C-fiber afferents could be the prevention of urinary incontinence and possibly the late onset damage to the upper urinary tract. Moreover, recent evidence in humans suggested that bladder afferents susceptible to capsaicin desensitization play a role in the development of autonomic dysreflexia in spinal cord injured patients (5). Autonomic dysreflexia is a medical emergency that can result in serious consequences such as a cerebral vascular accident. Therefore, early prevention of this condition makes the clinical application of prophylactic vanilloid therapy more attractive.

We have found that a single capsaicin dose can suppress uninhibited detrusor contractions up through 34 days in chronic SCI animals (Fig. 4). To our knowledge, this is the first time to specifically show the duration of NDO suppression in the bladders of spinalized rats after capsaicin injection using an open CMG. Igawa *et al.* (5) observed the effect of capsaicin lasting 3–12 months after a single intravesical instillation in SCI patients. Early capsaicin administration also showed NDO suppression 4 weeks after injury in our animals. These results indicate that prophylactic application of vanilloid therapy to acute SCI patients before C-fiber afferents become hyperexcitable may prevent the development of NDO and possibly autonomic dysreflexia and that only intermittent reapplication every few months could be necessary for its continued effect.

Acknowledgements

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