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Voiding Reflex in Chronic Spinal Cord Injured Cats Induced by Stimulating and Blocking Pudendal Nerves

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Abstract

Aims—To induce efficient voiding in chronic spinal-cord-injured (SCI) cats.

Methods—Voiding reflexes induced by bladder distension or by electrical stimulation and block of pudendal nerves were investigated in chronic SCI cats under α -chloralose anesthesia.

Results—The voiding efficiency in chronic SCI cats induced by bladder distension was very poor compared to that in spinal intact cats ($7.3 \pm 0.9\%$ vs. $93.6 \pm 2.0\%$, $P < 0.05$). In chronic SCI cats continuous stimulation of the pudendal nerve on one side at 20 Hz induced large amplitude bladder contractions, but failed to induce voiding. However, continuous pudendal nerve stimulation (20 Hz) combined with high-frequency (10 kHz) distal blockade of the ipsilateral pudendal nerve elicited efficient ($73.2 \pm 10.7\%$) voiding. Blocking the pudendal nerves bilaterally produced voiding efficiency ($82.5 \pm 4.8\%$) comparable to the efficiency during voidings induced by bladder distension in spinal intact cats, indicating that the external urethral sphincter (EUS) contraction was caused not only by direct activation of the pudendal efferent fibers, but also by spinal reflex activation of the EUS through the contralateral pudendal nerve. The maximal bladder pressure and average flow rate induced by stimulation and bilateral pudendal nerve block in chronic SCI cats were also comparable to those in spinal intact cats.

Conclusions—This study shows that after the spinal cord is chronically isolated from the pontine micturition center, bladder distension evokes a transient, inefficient voiding reflex, whereas stimulation of somatic afferent fibers evokes a strong, long duration, spinal bladder reflex that elicits efficient voiding when combined with blockade of somatic efferent fibers in the pudendal nerves.

Keywords

spinal cord injury; cat; voiding; pudendal nerve; stimulation; block

INTRODUCTION

Voiding is dependent on the coordinated actions of the bladder and EUS under the control of brain and lumbosacral spinal cord (Barrington 1931, 1941; de Groat et al. 1993). During voiding, the bladder contracts and the EUS relaxes. However, after SCI above lumbosacral level this coordinated action between bladder and EUS disappears (de Groat et al. 1993). Instead, bladder and EUS contract simultaneously (termed detrusor sphincter dyssynergia), which generates high bladder pressures, prevents complete elimination of urine, and requires daily urethral catheterization (Burns et al. 2001; de Groat et al. 1993). High bladder pressure can cause vesicoureteral reflux and renal failure in the long-term. Residual urine in the bladder and frequent urethral catheterization can cause infection (Burns et al. 2001; Jamil 2001). In the

1970s Brindley and co-workers developed an implantable sacral anterior root stimulator to restore voiding function after SCI. This system is now commercially available (Finetech Medical Limited, Wellyn Garden City, UK) and has been implanted in over 2000 people with SCI around the world (Brindley 1977,1994;Brindley and Rushton 1990;Creasey 1993;Jamil 2001;van Kerrebroeck 1996). It requires sacral posterior root rhizotomy to prevent detrusor sphincter dyssynergia in order to achieve the optimal result (Brindley 1994;Creasey 1993;van Kerrebroeck 1996). Although Brindley's method is the most successful treatment currently available to restore micturition after SCI, the sacral posterior root rhizotomy also disrupts reflex sexual and defecation functions and is irreversible (Brindley 1994;Brindley and Rushton 1990;Creasey 1993).

It is well known that stimulation of the pudendal nerve or a branch of this nerve at a frequency below 10 Hz can inhibit reflex bladder activity in both animals and humans with or without SCI (Vodusek et al. 1986,1988;Walter et al. 1993;Mazieres et al. 1997;Sundin et al. 1974;Fall et al. 1978;Lindstrom et al. 1983). This inhibitory pudendal-to-bladder reflex has been used to suppress neurogenic detrusor overactivity after SCI (Kirkham et al. 2001; Previnaire et al. 1996,1998;Vodusek et al. 1986). However, bladder excitation by stimulation of pudendal afferent axons should also be possible because mechanical stimulation of the perigenital area in chronic SCI cats or dogs can induce bladder contractions (de Groat 1975;Walter et al. 1989). This excitatory perineal-to-bladder reflex, which is also present in neonatal kittens (de Groat et al. 1975;de Groat 2002; Thor et al. 1986,1990) but suppressed during development, reappears after chronic SCI (de Groat and Ryall 1969;de Groat 1975;de Groat et al. 1993).

The response to mechanical stimulation of the perigenital region must be due in part to activation of pudendal afferent pathways since the pudendal nerve provides innervation to this skin area. Direct pudendal nerve stimulation elicits reflex efferent firing in the pelvic nerve (de Groat et al. 1981; Mazieres et al. 1997), reflex bladder contractions (Thor et al. 1990) and voiding (Boggs et al. 2006) in spinal cord intact cats. It also induced small bladder contractions in acute SCI cats (Boggs et al. 2005; Shefchyk and Buss 1998). In chronic SCI cats, pudendal nerve stimulation evoked short latency excitatory postsynaptic potentials in bladder parasympathetic preganglionic neurons (de Groat and Ryall 1969) and induced firing in bladder postganglionic nerves (de Groat et al. 1981). More recently, bladder excitation was demonstrated by intraurethral electrical stimulation in humans with complete SCI (Gustafson et al. 2003, 2004), in which the urethral sensory nerve fibers (one branch of pudendal afferents) were assumed to be activated.

Our recent studies (Tai et al. 2006a,b) revealed that the pudendal-to-bladder spinal reflex in chronic SCI cats is frequency-dependent. It is inhibitory at frequencies below 10 Hz, but excitatory at a frequency of 20 Hz (Tai et al. 2006a), and efficient post-stimulus voiding could be induced by intermittent 20 Hz pudendal nerve stimulation (Tai et al. 2006b). The present study further determined if an efficient voiding reflex can be induced in the chronic SCI cats by a continuous 20 Hz pudendal nerve stimulation. We used a nerve blocking technique to determine if detrusor sphincter dyssynergia interferes with pudendal nerve stimulation-induced voiding. Our previous studies (Tai et al. 2004,2005) revealed that electrical stimulation of the pudendal nerve in cats at a frequency of 6-10 kHz blocks nerve conduction. In this study, combining excitatory and blocking stimulation of the pudendal nerves induced an efficient voiding reflex in chronic SCI cats.

MATERIALS and METHODS

All protocols involving the use of animals in this study were approved by the Animal Care and Use Committee at the University of Pittsburgh.

A total of 6 female cats were used in this study (3 spinal intact and 3 chronic SCI animals, 3.5-4.5 kg). For the 3 chronic SCI cats that were also used in our previous study (Tai et al. 2006b), spinal cord transection was performed (3-11 months prior to the experiment) at the T9-T10 spinal cord level by a dorsal laminectomy under isoflurane anesthesia and aseptic conditions. The procedures for spinal cord transection and animal care have been described elsewhere (Tai et al. 2006a,b). During the experiments, animals (both spinal intact and SCI) were anesthetized with α -chloralose (60 mg/kg i.v., supplemented as needed) following induction with halothane (2-3% in O₂). Systemic blood pressure was monitored via a cannula placed in the right carotid artery. A tracheotomy was performed and a tube was inserted to secure the airway. A catheter for i.v. infusion was introduced into right ulnar vein. A double lumen catheter (5 French) was inserted into the bladder via the dome and secured by a ligature (see Fig.1A). One lumen of the catheter was attached to a pump to infuse the bladder with saline, and the other lumen was connected to a pressure transducer to monitor the bladder activity. A funnel was used to collect the voided fluid in a beaker that was attached to a force transducer to record the volume. For the 3 chronic SCI animals, the pudendal nerves were accessed posteriorly between the sciatic notch and the tail. Two tripolar cuff electrodes [Micro Probe, Inc., NC223(Pt)] were placed around the left pudendal nerve (Elec. #1 and #2 in Fig. 1A). A third tripolar cuff electrode was placed around the right pudendal nerve (Elec. #3 in Fig.1A). The 3 electrode leads in each cuff electrode were made of platinum wires (diameter 0.01 mm) with a 2 mm distance between the leads. The two leads at each end of the cuff electrode were connected together (see Fig.1A). After implanting the pudendal nerve electrodes, the muscle and skin were closed by sutures. For the 3 spinal intact animals, electrodes were not implanted on the pudendal nerve. The temperature of the animals was maintained at 35-37°C during the experiments using a heating pad. A pulse oximeter (Nonin Medical, Inc., 9847V) with its sensor clipped on the tongue of animals was used to monitor the arterial oxygen saturation and heart rate. Blood pressure, heart rate and front paw withdraw reflex were used to evaluate the anesthetic depth.

In the experiments in chronic SCI cats, uniphasic pulses at 20 Hz frequency, 2-10 V intensity and 0.2 ms pulse width were used to stimulate the pudendal nerve at electrode #1 (see Fig.1B) in order to induce the excitatory pudendal-to-bladder reflex and bladder contractions. Our previous study (Tai et al. 2006) in chronic SCI cats showed that 20 Hz pudendal nerve stimulation induces strong bladder contractions. The stimulation intensity was determined at the beginning of each experiment by a preliminary test of its effectiveness to induce bladder contractions. In order to block pudendal nerve conduction and prevent an EUS contraction, a train of high-frequency, biphasic, continuous (duty cycle 100%), charge-balanced, rectangular pulses at 10 kHz frequency and 10 mA intensity were delivered to electrode #2 and/or #3 (see Fig.1B). The stimulation frequency and intensity were shown in our previous studies (Tai et al. 2004,2005) to be effective in blocking the pudendal nerves of cats. A Grass S88 stimulator (Grass Medical Instruments) with stimulus isolator (Grass Medical Instruments, SIU5) was used to generate the uniphasic stimulus pulses for electrode #1. The high-frequency, biphasic stimulation waveforms (10 kHz) used at electrodes #2 and #3 were generated by a computer with a digital-to-analog circuit board (National Instruments, AT-AO-10) that was programmed using LabView programming language (National Instruments). Linear stimulus isolators (World Precision Instruments, A395) were used to deliver the high-frequency, biphasic constant current pulses to the nerves via electrodes #2 and #3.

Starting with the bladder empty, saline was slowly infused (0.5-4 ml/min) into the bladder to induce a voiding reflex (i.e. a cystometrogram – CMG). Bladder capacity was defined as the infused volume at which a bladder contraction was induced and fluid was released from the urethral orifice. When fluid was released, the infusion was stopped. The distension induced voiding was evaluated in both spinal intact and chronic SCI cats. In the chronic SCI cats during an intercontraction quiet period after stopping the bladder infusion, 20 Hz stimulation was

applied to the pudendal nerve to induce bladder contractions. In another stimulation paradigm, prior to the start of 20 Hz stimulation at electrode #1, the 10 kHz blocking stimulation was applied either to electrode #2 only or to electrodes #2 and #3 (see Fig. 1B) in order to block the EUS contraction during the 20 Hz pudendal nerve stimulation. Voiding efficiency, maximal bladder pressure, and average flow rate were measured in order to evaluate the effectiveness of the induced voiding reflex. Voiding efficiency is defined as the total voided volume divided by the total infused volume. Parameters measured from multiple trials in the same animal were averaged and then presented as mean $\pm$ standard error (SE). Since ANOVA analysis showed no significant difference between trials in the same animals, two-way ANOVA (experimental conditions versus animals) was used to determine any statistical significance ($P < 0.05$). Since each parameter was measured multiple times in the same animal, for each experimental condition there were 3 data sets (mean, SD, and N) from the 3 animals. Two-way ANOVA was performed on the 3 data sets (animals) for different experimental conditions (control, nerve stimulation and block, etc.).

RESULTS

Voiding reflex in normal and chronic SCI cats

As shown in Fig. 2, the cystometrograms in spinal intact and chronic SCI cats were markedly different. During bladder filling in chronic SCI cats the bladder exhibited multiple, low amplitude, short duration, non-voiding contractions (i.e., neurogenic detrusor overactivity, Fig. 2B); whereas the bladder of spinal cord intact animals was quiescent until the onset of voiding (Fig. 2A). Voiding was also different. Compared to the bladder contractions induced by bladder distension in spinal cord intact cats the contractions induced by distension in chronic SCI cats were weaker and considerably less efficient in producing voiding (Fig. 2A and B). On average the peak intravesical pressures during reflex contractions in chronic SCI cats were only 30% of those in spinal intact cats (Fig. 3A, 23.1 ± 1.7 cmH₂O vs. 72.5 ± 11.8 cmH₂O, $P < 0.05$). The average duration of contractions was only 20% of the duration in spinal intact animals (21.9 ± 0.9 seconds vs. 109.3 ± 8.2 seconds, $P < 0.05$). Average voiding efficiency ($7.3 \pm 0.9\%$, Fig. 4) and flow rate (0.23 ± 0.07 ml/s, Fig. 3B) were markedly ($P < 0.05$) lower than the values in spinal intact cats ($93.6 \pm 2.0\%$ and 0.56 ± 0.16 ml/s respectively, see Fig. 3B and Fig. 4). Note that in both types of animals the saline infusion was stopped when fluid was released from the urethral meatus.

To determine if voiding might improve as bladder volume increased with continued infusion in chronic SCI cats the cystometrograms were also continued beyond the time of the first void (Fig. 5A). This only produced a series of short duration, small bladder contractions of approximately the same amplitude and duration. Each contraction released only a small amount of fluid equivalent to the volume infused during each contraction interval. During the experiment shown in Fig. 5A the residual bladder volume remained static during the series of small voiding reflexes. At the end of 12 min infusion beyond the first void, the residual bladder volume was almost equal to the volume (19 versus 20 ml) prior to the first void. Thus in chronic SCI cats continued infusion of fluid did not increase bladder volume; and voiding efficiency during each void was still poor.

A second approach to improve voiding efficiency was to increase bladder volume prior to voiding utilizing a low frequency pudendal nerve stimulation (3 Hz) which we showed in a previous study (Tai et al. 2006) suppressed non-voiding contractions during bladder filling and increased bladder capacity in chronic SCI cats. It was anticipated that at a larger bladder volume a more prolonged and larger amplitude bladder contraction might occur. However as shown in Fig. 5B in the same chronic SCI cat used in Fig. 2B, 3 Hz pudendal nerve stimulation inhibited bladder activity during filling and increased bladder capacity from 74 ml to 124 ml,

but after the stimulation was stopped only short duration bladder contractions occurred and voiding was still inefficient (31 ml voided leaving 93 ml residual volume in the bladder).

Voiding reflex in chronic SCI cats induced by pudendal nerve stimulation and block

As shown in Fig. 6, 20 Hz excitatory pudendal nerve stimulation applied to electrode #1 (see Fig.1) elicited large amplitude (40 cm H₂O), long duration (80-100 sec) bladder contractions in a chronic SCI cats that were equivalent to the voiding contractions in cats with an intact spinal cord. However, voiding did not occur during the 20 Hz stimulation. On the other hand, when 10 kHz blocking stimulation was applied for a brief period (20 sec) to the pudendal nerves bilaterally (electrodes #2 and #3, see Fig.1) during the 20 Hz excitatory stimulation, the bladder pressure immediately decreased and voiding occurred (Fig. 6). When the blocking stimulation was stopped, voiding stopped and bladder pressure increased. The bladder pressure was maintained until the end of 20 Hz stimulation when an additional void occurred presumably due to the rapid relaxation of the urethral sphincter while the bladder pressure was still high.

An efficient voiding reflex was also induced in chronic SCI cats by the 20 Hz pudendal nerve stimulation when the 10 kHz blocking stimulation was applied ipsilaterally (i.e. only applied to the electrode #2, see Fig.1) or bilaterally (i.e. applied to both electrodes #2 and #3, see Fig. 1) prior to the excitatory pudendal nerve stimulation. Fig. 7A shows in a chronic SCI cat that a large amount (53 ml) of fluid was voided from a bladder containing 86 ml when the 20 Hz stimulation was combined with the ipsilateral 10 kHz blocking stimulation resulting in a voiding efficiency of 61.6%. The voiding occurred primarily during the first stimulation period when two periods of stimulation were used. Fig. 7B shows in another chronic SCI cat that the 20 Hz stimulation combined with bilateral 10 kHz blocking stimulation also induced an efficient (88%) voiding reflex. As shown in Fig. 7B, a small amplitude bladder contraction was induced at the onset of the 10 kHz blocking stimulation without voiding. When the 20 Hz stimulation was started, the bladder pressure increased quickly and then decreased once fluid started to flow through the open urethra. During the induced voiding the fluid flow was a steady stream and the bladder pressure was relatively low (less than 30 cmH₂O).

On average the 20 Hz pudendal nerve stimulation coupled with 10 kHz ipsilateral block significantly ($P<0.05$) increased the voiding efficiency in chronic SCI cats to $73.2\pm10.7\%$ compared to the voiding efficiency induced by bladder distension ($7.3\pm0.9\%$) as shown in Fig. 4. With 10 kHz bilateral block of the pudendal nerves, the 20 Hz pudendal nerve stimulation further increased voiding efficiency to $82.5\pm4.8\%$. This efficiency is not significantly different from the voiding efficiency in spinal intact cats induced by bladder distension, although the voiding efficiency induced by ipsilateral block is still significantly different (Fig.4). As shown in Fig. 3A, the maximal bladder pressure (31.4 ± 6.4 cmH₂O) during voiding induced by the 20 Hz pudendal nerve stimulation with 10 kHz bilateral nerve block was significantly ($P<0.05$) increased in chronic SCI cats compared to the bladder distension induced voiding (23.1 ± 1.7 cmH₂O). But it was still significantly ($P<0.05$) lower than that in spinal intact cats (72.5 ± 11.8 cmH₂O). Therefore, simultaneously stimulating and blocking the pudendal nerves induced low pressure voiding in chronic SCI cats. The average flow rate (0.68 ± 0.08 ml/s) was significantly ($P<0.05$) increased by the 20 Hz pudendal nerve stimulation with bilateral 10 kHz block in chronic SCI cats compared to the distension induced voiding (0.23 ± 0.07 ml/s) (see Fig. 3B). It was not significantly different ($P>0.05$) from the flow rate in spinal intact cats (0.56 ± 0.16 ml/s). Therefore, in chronic SCI cats 20 Hz pudendal nerve stimulation combined with 10 kHz nerve block induced a markedly different voiding reflex than bladder distension (see Fig. 2B, Fig. 5 and Fig.7) resulting in an efficient voiding (Fig.4) with a fast flow rate (Fig. 3B) and a low bladder pressure (Fig. 3A).

DISCUSSION

This study revealed marked differences in voiding reflexes in chloralose anesthetized cats with an intact spinal cord and in chronic SCI cats (Fig. 2-5). Chronic SCI cats exhibited a reduced voiding efficiency and urethral flow rate that is attributable to low amplitude and short duration reflex bladder contractions as well as to poor coordination between the urinary bladder and the EUS. Electrical stimulation of afferent axons in the pudendal nerve reversed the defect in bladder contractions, eliciting large amplitude, long duration, reflex bladder contractions but still did not improve voiding efficiency due to simultaneous activation of motor pathways to the EUS (Fig. 6). However when the motor pathways were blocked by high frequency stimulation (10 kHz) of the pudendal nerves unilaterally or bilaterally, voiding efficiency markedly improved (Fig.4 and Fig.7). These results raise the possibility that combined pudendal afferent nerve stimulation and efferent nerve block might be useful in promoting voiding in people with SCI.

Voiding reflex in normal cats vs. voiding reflex in chronic SCI cats

In normal cats, the pontine micturition center (PMC) located at the rostral pons coordinates bladder and EUS activity during voiding (Barrington 1931,1941;de Groat et al. 1993). During bladder filling when afferent input to the PMC reaches the threshold for triggering micturition (i.e. bladder capacity) descending projections from the PMC induce a sustained bladder contraction with a simultaneous EUS relaxation resulting in release of a large volume of fluid from the bladder (see Fig.2 A). This spinobulbospinal voiding reflex produces a fast flow rate (Fig.3 B) with a high voiding efficiency (Fig.4). However, in chronic SCI cats the contribution of the PMC is lost and voiding is mediated purely by a spinal reflex. This spinal voiding reflex can only induce a series of small, short-lasting bladder contractions (Fig.2 B, Fig.3 A and Fig. 5) when the bladder volume reaches a threshold level, but only a very small percentage of bladder volume (Fig.4) is released at a slow flow rate (Fig.3 B).

The inefficiency of voiding in chronic SCI cats might be attributable to two different spinal reflexes. One is the bladder-to-bladder spinal reflex, and the other is the bladder-to-pudendal spinal reflex. The bladder-to-bladder spinal reflex is brief (see Fig.2 B and Fig.5) and generates smaller bladder pressures (see Fig.3 A) compared to the spinobulbospinal reflex in spinal intact cats. In awake, chronic SCI cats (Walter et al. 1997) similar short duration, small amplitude bladder contractions were also observed during voiding. This indicates that the bladder-to-bladder spinal reflex is very different from the bladder-to-bladder supraspinal reflex mediated by the PMC. Once it is triggered, the supraspinal reflex can maintain a large amplitude bladder contraction and sustain the bladder pressure as the bladder volume becomes smaller during the voiding (Fig.2 A). This sustained bladder contraction is driven by a constant input from the PMC to the parasympathetic neurons in the sacral spinal cord (de Groat and Ryall 1969;de Groat et al. 1993). However, the bladder-to-bladder spinal reflex lacks of this sustained input. Instead, the bladder-to-bladder spinal reflex is directly driven by the tension receptors in the bladder wall. Once voiding occurs, and bladder volume and tension in the bladder wall are reduced, attenuation of afferent input to spinal cord seems to turn off the reflex (Fig.2 B and Fig.5). Therefore, the bladder-to-bladder spinal reflex can not sustain the bladder contraction as the bladder volume declines during voiding. In addition to the weak bladder-to-bladder spinal reflex, voiding inefficiency in chronic SCI cats is also caused by a bladder-to-pudendal spinal reflex (Blaivas 1982;Shefchyk 2006). This reflex triggers EUS contractions and increased urethral outlet resistance during a bladder contraction (i.e. detrusor sphincter dyssynergia), which further reduced the ability of the distension induced, small, transient bladder contractions to eliminate fluid from the bladder (see Fig.2 B and Fig.5).

Although it seems unlikely that the surgical manipulation on the pudendal nerves caused the small, transient bladder contractions in the chronic SCI cats, it is worth noting that the pudendal

nerves in normal animals were not surgically manipulated and this might have contributed to the differences in bladder activity between normal and SCI cats. Thus the factors contributing to the differences between the bladder-to-bladder spinal reflex in chronic SCI cats and the bladder-to-bladder spinobulbospinal reflex in normal cats need to be further investigated.

Pudendal-to-bladder spinal reflex

In this study we have demonstrated that an excitatory pudendal-to-bladder spinal reflex exists in chronic SCI cats. This spinal reflex can generate sustained bladder contractions (Fig.6) strong enough to induce efficient voiding if the EUS contraction can be prevented (Fig.4 and Fig.7). The 20 Hz pudendal nerve stimulation provides a sustained excitatory input to the sacral parasympathetic neurons (de Groat and Ryall 1969) that is similar to what is provided by the PMC. However, it appears that this excitatory pudendal-to-bladder spinal reflex is also dependent on bladder volume. At a smaller bladder volume, it becomes weaker (see Fig.6). Thus it is likely that there is a positive interaction between pudendal and bladder afferent inputs to the spinal micturition reflex circuitry. The excitatory pudendal-to-bladder spinal reflex could be induced during distal blockade of pudendal nerve (see Fig.7) indicating that this excitatory spinal reflex is activated by stimulating the afferent fibers in the pudendal nerve rather than the efferent fibers.

The pudendal-to-bladder spinal reflex in chronic SCI cats can be either excitatory or inhibitory depending on the stimulation frequency (Tai et al. 2006). Previous studies (Walter et al. 1993; Mazieres et al. 1997; Sundin et al. 1974; Fall et al., 1978; Lindstrom et al. 1983) showed in both normal and SCI cats that an inhibitory pudendal-to-bladder reflex was induced by pudendal nerve stimulation at frequency below 10 Hz (see also Fig.5 B). However, as demonstrated in this and our previous study (Tai et al. 2006), the pudendal-to-bladder spinal reflex in chronic SCI cats becomes excitatory at a stimulation frequency of 20 Hz. Although the mechanism of this frequency dependence is unknown, it is clear that different stimulation frequencies must activate different spinal micturition reflex circuitry. Barrington (1931, 1941) identified a spinal reflex from urethra to bladder when urine flows through the urethra. He showed that this reflex can induce a bladder contraction when bladder volume is high. Meanwhile, Garry et al. (1959) reported that fluid flowing through the urethra could inhibit bladder activity when bladder volume was low. Since the urethra is innervated by the pudendal nerve, pudendal nerve stimulation at different stimulation frequencies might trigger the different urethra-to-bladder reflexes.

In acute SCI cats (i.e. within hours after spinal cord transection), pudendal nerve stimulation induced small (less than 20 cmH₂O) bladder contractions (Boggs et al. 2005; Shefchyk and Buss 1998). However, the pudendal-to-bladder spinal reflex in acute SCI cats is excitatory regardless of the frequency of pudendal nerve stimulation (Boggs et al. 2005), which is very different from the effects in chronic SCI cats. This difference between acute and chronic SCI cats may be due to the fact that neuroplasticity in the spinal cord which underlines the emergence of excitatory bladder-to-bladder and pudendal-to-bladder spinal reflexes requires several weeks to fully develop. Neurogenic detrusor overactivity and detrusor sphincter dyssynergia that are indicators of spinal reorganization after SCI exist in chronic SCI animals and humans, but do not exist after acute SCI. Instead, acute SCI results in detrusor areflexia (i.e. loss of reflex bladder contractions), which may explain why only the excitatory pudendal-to-bladder reflex could be observed in acute SCI cats (Boggs et al. 2005).

A recent study (Boggs et al., 2006) also showed that intermittent pudendal nerve stimulation at 33 Hz induced a voiding reflex in cats with an intact spinal cord. However, it is difficult to attribute this voiding solely to a spinal reflex in spinal intact cats, since the spinobulbospinal micturition reflex is intact and the PMC coordinates voiding once the bladder contraction is initiated by pudendal nerve stimulation. In addition pudendal nerve stimulation evokes a long

latency reflex discharge on bladder postganglionic nerves in spinal intact cats, which is presumably mediated by a spinobulbospinal pathway because it is eliminated in chronic SCI cats (de Groat and Ryall 1969). Therefore, an efficient voiding reflex is expected to be induced in spinal intact cats by pudendal nerve stimulation just like the voiding reflex induced by bladder distension in spinal intact cats (see Fig.2 A and Fig.4).

EUS contractions induced by 20 Hz pudendal nerve stimulation

The urethral outlet resistance in the chronic SCI cats could be generated by three different mechanisms of EUS activation. The first one is due to the direct activation of the pudendal efferent input to the EUS by the 20 Hz pudendal nerve stimulation. This could cause a strong EUS contraction that blocks voiding even during a large amplitude bladder contraction (see Fig.6). The second type of EUS contraction is due to the excitatory bladder-to-pudendal spinal reflex (i.e. detrusor sphincter dyssynergia) (Blaivas 1982; de Groat et al. 1993). This spinal reflex not only contributes to the low voiding efficiency in the chronic SCI cats induced by bladder distension (see Fig.2 B and Fig.5), but also plays a role in inducing EUS contractions during the bladder contractions induced by 20 Hz pudendal nerve stimulation. The third type of EUS contraction is due to the excitatory pudendal-to-pudendal spinal reflex (Chang et al. 2006; Shefchyk 2006; Thor et al. 1989). This spinal reflex which is distributed bilaterally could cause EUS contractions via the contralateral pudendal nerve even when the ipsilateral pudendal nerve is blocked. Compared to ipsilateral block, bilateral block of the pudendal nerves further increased voiding efficiency to a level that is not significantly different from spinal intact cats (see Fig.4). This shows that the EUS contraction was partially induced by reflex efferent activity in the contralateral pudendal nerve. Due to the three types of EUS contractions induced during 20 Hz pudendal nerve stimulation, voiding efficiency was maximal during simultaneous block of the pudendal nerves bilaterally (see Fig.4).

A previous study (Sawan et al. 1996) in chronic SCI dogs claimed that complete bladder emptying could be achieved without dorsal rhizotomy by stimulating the sacral spinal roots at frequencies of 300-350 Hz to fatigue the EUS. However, another study (Ishigooka et al. 1994) showed that pudendal nerve stimulation between 100 Hz and 1000 Hz could only fatigue the EUS by 30-45% since the majority of the EUS muscles are slow twitch fibers that are fatigue resistant. Further, fatigue stimulation becomes gradually less effective for long-term use since the sphincter could change to become more fatigue resistant (Schmidt 1983).

Safety of the high-frequency blocking stimulation

Although the effectiveness of biphasic, high frequency (10 kHz), charge-balanced, electrical stimulation of the pudendal nerves has been demonstrated in this and previous studies (Tai et al. 2004, 2005), it remains to be determined if this stimulation is safe for long-term use. It is known that biphasic, charge-balanced, electrical pulses will cause less damage to the nervous tissue than uniphasic pulses (Agnew and McCreedy 1990), but the long-term use of this nerve blocking method needs to be evaluated in animal studies before testing in humans. Acute damage of the pudendal nerve caused by high-frequency biphasic stimulation seems unlikely since our previous study (Tai et al. 2005) in animals showed that this blocking stimulation applied repetitively (1 minute stimulation every 1-3 minutes) during a period of 43 minutes did not alter the neurally evoked EUS response. The potential human application of the high-frequency nerve blocking method will be limited to 3-5 times a day for 1-3 minutes each time to induce voiding in SCI people. The risk of nervous tissue damage is low when the nerve is only stimulated for a short time during 24 hours (Agnew and McCreedy 1990).

Potential human application

The effectiveness of excitatory 20 Hz pudendal nerve stimulation needs to be tested in humans to assess the potential clinical benefits. Intermittent voiding responses in quadruped animals

is associated with squirting of urine and territorial marking (Walter et al. 1989,1993,1997), which is different from voiding in humans that occurs as a steady stream of urine. Therefore, the pudendal-to-bladder reflex after SCI might be different in cats and humans. However, intraurethral electrical stimulation at a frequency of 20 Hz excited the bladder in people with complete SCI (Gustafson et al. 2003,2004). Since the urethra is innervated by pudendal nerve, this indicates that 20 Hz stimulation might be also effective in activating the bladder in humans.

Inducing a voiding reflex after SCI by pudendal nerve stimulation and block would not require sacral posterior root rhizotomy, thereby improving on Brindley's method (Brindley ¹⁹⁷⁷, 1994; Brindley and Rushton 1990; Creasey 1993; van Kerrebroeck 1996) by preserving the spinal reflexes for bowel, bladder, and sexual functions in people with SCI. Meanwhile, eliminating the requirement of sacral posterior root rhizotomy also provides hope for people with SCI to benefit from any advance in neural regeneration and repair techniques in the future (Bulsara et al. 2002; Blesch et al. 2002). Spinal surgery that is needed in Brindley's method to access the spinal roots would not be necessary either, because only the pudendal nerves would be exposed to implant stimulating electrodes. Compared to spinal surgery, the pudendal nerves can be more easily accessed with minimal surgery (Schmidt 1989; Spinelli et al. 2005).

CONCLUSIONS

In summary, our studies have revealed that significant differences exist between the spinal micturition reflex in chronic SCI cats and the spinobulbospinal micturition reflex in spinal intact cats. In addition voiding is very inefficient in chronic SCI cats as in SCI people. The improvement in voiding induced by pudendal nerve stimulation and block in chronic SCI cats provided further evidence indicating the feasibility of a new neuroprosthetic device to restore micturition function in people after SCI.

Acknowledgments

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A. Experimental Setup

B. Pudendal Nerve Stimulation and Block

Fig.1.

A. Experimental setup. B. Pudendal nerve stimulation (20 Hz) and block (10 kHz) delivered to each electrode. The 20 Hz stimulation at electrode #1 activates an excitatory pudendal-to-bladder spinal reflex to induce bladder contraction, while the 10 kHz stimulations at electrodes #2 and #3 block pudendal nerve conduction bilaterally to prevent EUS contraction. EUS – External Urethral Sphincter.

Fig.2.

Voiding reflex induced by bladder distension in a normal (A) and a chronic SCI (B) cat. A: In a normal cat, the bladder was infused at 0.5 ml/min. At the infusion stop a total of 21 ml was infused, and 20 ml was voided with a voiding efficiency of 95.2%. B: In a chronic SCI cat (11 months after SCI), the bladder was infused at 4 ml/min. At the infusion stop a total of 74 ml was infused, but only 4 ml was voided with a voiding efficiency of 5.4%.

Fig.3.

Maximal bladder pressure (A) and average flow rate (B) during voiding in spinal intact and chronic SCI cats (N=3). Spinal intact – during voiding induced by bladder distension in spinal intact cats (see Fig.2 A). Control – during voiding induced by bladder distension in chronic SCI cats (see Fig.2 B). 20Hz+block2 – during voiding induced by 20 Hz stimulation of the left pudendal nerve with 10 kHz blocking of both left and right pudendal nerves in chronic SCI cat (see Fig.7 B). * indicates statistical significance ($P<0.05$).

Fig.4.

Voiding efficiency in spinal intact and chronic SCI cats (N=3). Spinal intact – efficiency of voiding induced by bladder distension in spinal intact cats (see Fig.2 A). Control – efficiency of voiding induced by bladder distension in chronic SCI cats (see Fig.2 B). 20Hz+block1 – efficiency of voiding induced by 20 Hz stimulation and 10 kHz blocking of the left pudendal nerve in chronic SCI cats (see Fig.7 A). 20Hz+block2 – efficiency of voiding induced by 20 Hz stimulation of the left pudendal nerve with 10 kHz blocking of both left and right pudendal nerves in chronic SCI cat (see Fig.7 B). * indicates statistical significance ($P<0.05$).

A. Continuous infusion

B. Larger bladder volume

Fig.5.

Voiding reflex induced by bladder distension in chronic SCI cats. A: In a chronic SCI cat (9 months after SCI), the bladder was continuously infused at 3 ml/min. At the first voiding contraction, a total of 20 ml was infused into the bladder. At the last voiding contraction, a total of 57 ml was infused, but a total of 38 ml was voided by a series of short-lasting bladder contractions resulting in 19 ml residual volume in the bladder. B: In the same chronic SCI cat as shown in Fig. 2 B, the bladder capacity was increased to 124 ml at the infusion stop by the inhibitory 3 Hz pudendal nerve stimulation. The black bar under the bladder pressure trace indicates the duration of the pudendal nerve stimulation. After stopping the 4 ml/min infusion and the stimulation, several short-lasting bladder contractions voided a total of 31 ml.

Fig.6.

Voiding reflex induced by 20 Hz pudendal nerve stimulation alone in a chronic SCI cat (9 months after SCI). The 10 kHz blocking stimulation was only briefly applied bilaterally to the pudendal nerves during the 20 Hz stimulation of the left pudendal nerve. 24 ml was infused into the bladder when the infusion was stopped. 12 ml was voided only during the blocking stimulation. The black bars on bladder pressure trace indicate the stimulation durations. Infusion rate: 2 ml/min.

Fig.7.

Voiding reflex induced by stimulating and blocking the pudendal nerves in chronic SCI cats. A: Both 20 Hz and 10 kHz stimulations were applied to the left pudendal nerve in a chronic SCI cat (11 month after SCI). 86 ml was infused into the bladder when the infusion was stopped. A total of 53 ml was voided with a voiding efficiency of 61.6%. Infusion rate: 4 ml/min. B: The 20 Hz stimulation was applied to the left pudendal nerve during 10 kHz bilateral blocking stimulation of the pudendal nerves in a chronic SCI cat (9 month after SCI). 25 ml was infused into the bladder when the infusion was stopped. 22 ml was voiding during the 20 Hz stimulation with a voiding efficiency of 88%. Infusion rate: 2 ml/min. The black bars on bladder pressure traces indicate the stimulation durations.