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Influence of Naloxone on Inhibitory Pudendal-to-Bladder Reflex in Cats

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Abstract

To determine the involvement of opioid receptors in the inhibitory pudendal-to-bladder reflex, the effect of naloxone (0.01–1 mg/kg, i.v.), an opioid receptor antagonist, on the inhibition of bladder activity evoked by pudendal nerve stimulation was investigated in α -chloralose anesthetized cats. The inhibition of reflex isovolumetric bladder contractions induced by pudendal nerve stimulation (5–10 Hz) at intensity threshold (T) for producing complete inhibition was significantly suppressed by naloxone at a high dose (0.3 mg/kg). However the inhibition elicited at higher intensities (1.5–3 T) was not changed. Naloxone (1 mg/kg) did not alter the frequency dependence of the inhibitory effect of pudendal stimulation. During cystometrograms (CMGs) pudendal nerve stimulation significantly increased bladder capacity to $155.1 \pm 24.5\%$ and $163.4 \pm 10\%$ of the control at stimulation intensities of 1 T and 1.5–3 T respectively. After administration of naloxone (1 mg/kg) the bladder capacity during pudendal nerve stimulation at inhibition threshold (1 T) was not significantly different from control, but it was significantly increased at higher intensities (1.5–3 T). Naloxone alone markedly reduced bladder capacity to $43 \pm 11.1\%$ of the control; and pudendal stimulation completely reversed this facilitatory effect. This study revealed that activation of opioid receptors contributes to or facilitates the inhibitory pudendal-to-bladder reflex. The reduction in bladder capacity after naloxone treatment also indicates that endogenous opioid peptides mediate a tonic inhibition of micturition. Understanding the neurotransmitter mechanisms involved in the inhibitory pudendal-to-bladder reflex could promote the development of new treatments for bladder overactivity and incontinence.

Keywords

opioid; neurotransmitter; neuromodulation; bladder; cats

INTRODUCTION

Sacral neuromodulation, which is elicited by stimulation of the S3 spinal nerve, is an FDA approved treatment for patients with refractory urgency, frequency, urge incontinence, and

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non-obstructive urinary retention (van Kerrebroeck et al., 2007; Nakib and Siegel, 2008; Oerlemans and van Kerrebroeck, 2008; Sutherland et al, 2007). However, pudendal neuromodulation has recently been reported to be superior to sacral neuromodulation in patients with intractable lower urinary tract symptoms (LUTS) (Peters et al., 2005, 2007). One possible explanation for this enhanced effectiveness is the recruitment of a broader segmental distribution of sacral afferent nerve fibers (S2–S4) with pudendal neuromodulation versus only S3 afferents for sacral neuromodulation. Understanding the mechanisms underlying pudendal neuromodulation could further promote its clinical application.

Recent animal studies demonstrated that pudendal neuromodulation is frequency dependent (Shen et al., 2008; Tai et al., 2006, 2008; Wang et al., 2009; Woock et al., 2008; Walter et al., 1992; Wheeler et al., 1993). In chronic spinal injured cats, 3–10 Hz pudendal nerve stimulation inhibited bladder activity, while 20 Hz stimulation excited the bladder (Tai et al., 2006, 2008; Wang et al., 2009). In spinal intact cats, transcutaneous stimulation of the pudendal nerve with a patch electrode attached to the skin overlying the pudendal nervemaximally suppressed bladder activity at 5–7 Hz; but at 20 Hz, the stimulation induced large amplitude bladder contractions (Shen et al., 2008). Similarly, 5–10 Hz stimulation of urethral or genital/penile afferent pathways projecting through the pudendal nerve inhibited distention-evoked bladder contractions (Walter et al., 1992; Wheeler et al., 1993), but stimulation at 33–40 Hz elicited bladder contractions (Woock et al., 2008). Currently, the mechanisms underlying frequency dependent pudendal neuromodulation are unknown.

The inhibitory pudendal-to-bladder reflex that promotes bladder storage is likely mediated by inhibitory neurotransmitters including glycine, GABA (gamma-aminobutyric acid), and enkephalins (i.e. opioid peptides) which have been shown to suppress bladder preganglionic neuron firing and reflex micturition in animals (Booth et al., 1985; de Groat, 1970; Dray and Metsch, 1984; Hisamitsu and de Groat, 1984; Maggi et al., 1987; Mazieres et al., 2006; Miyazoto et al., 2003, 2004; Noto et al., 1991; Pehrson et al., 2002; Roppolo et al., 1983; Su et al., 1997; Thor et al, 1983). Intrathecal administration of glycine suppresses bladder activity (Miyazoto et al., 2003, 2004), while administration of strychnine (a glycine receptor antagonist) induces bladder contractions (Miyazoto et al., 2004). GABA, muscimol (GABA_A receptor agonist) and baclofen (GABA_B receptor agonist), administered intravenously, intrathecally, or intracerebroventrically, also inhibit the micturition reflex (Maggi et al., 1987; Pehrson et al., 2002). Similar inhibitory responses are elicited by enkephalins and other opioid receptor agonists given intravenously, intrathecally, or intracerebroventricularly (Booth et al., 1985; Dray and Metsch, 1984; Hisamitsu and de Groat, 1984; Noto et al., 1991; Roppolo et al., 1983; Su et al., 1997; Thor et al, 1983). Naloxone (an opioid receptor antagonist) can block these inhibitory responses and excite the bladder (Booth et al., 1985; Hisamitsu and de Groat, 1984; Noto et al., 1991; Roppolo et al., 1983; Thor et al, 1983). Based on these pharmacological studies, it seems reasonable to hypothesize that inhibitory control of the bladder probably involves several neurotransmitters (Fowler et al., 2008).

The goal of this study was to determine the involvement of enkephalins in the inhibitory pudendal-to-bladder reflex by administering naloxone during pudendal neuromodulation. Understanding the neurotransmitter mechanisms underlying pudendal neuromodulation might promote the development of new pharmacological treatments for patients suffering from LUTS, or further improve the clinical outcome by combining neuromodulation with drug therapy.

MATERIALS AND METHODS

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

Experimental Setup

Experiments were conducted in 10 female cats (2.5–3.9 kg) anesthetized initially with isoflurane (2–5% in oxygen) and maintained with α -chloralose (65 mg/kg i.v. with supplementation as necessary). Heart rate and blood oxygen level were measured by a pulse oximeter (9847V, NONIN Medical, Inc., Plymouth, MN) with the sensor attached to the tongue. Systemic blood pressure was measured via a catheter in the carotid artery. Drug and fluid were administered via the ulnar vein, and airway access was secured with a tracheostomy tube.

The ureters were isolated via an abdominal incision and drained externally. The bladder was cannulated through the urethra with a double lumen catheter. One lumen was used to infuse saline into the bladder and the other was attached to a pressure transducer to record the bladder pressure. A ligature was tied around the urethra to prevent leakage. The pudendal nerve was dissected (usually from the left side) via a 3–4 cm incision between the tail and the sciatic notch. A tripolar cuff electrode (NC223pt, MicroProbe, Inc, Gaithersburg, MD) was applied around the nerve and connected to a stimulator (S88, Grass Medical Instruments, Quincy, Massachusetts).

Stimulation Protocol

In order to induce isovolumetric rhythmic bladder contractions, the bladder was first infused with saline to a volume between 100–110% of the bladder capacity that is defined as the threshold bladder volume to induce a micturition contraction. At this bladder volume, stimulus trains of uniphasic rectangular pulses (0.1 ms pulse width) at different frequencies (1–40 Hz) were delivered to the pudendal nerve through the cuff electrode to determine the effective frequencies for inhibiting bladder activity. The stimulation duration was always longer than the period of at least two bladder contractions in order to confirm the inhibitory effect. Based on our previous studies (Shen et al., 2008; Tai et al., 2006, 2008; Wang et al., 2009), 5 Hz stimulation was used to determine the intensity threshold (T) for completely inhibiting the isovolumetric bladder contractions. Then, multiple times (1–3 T) the threshold intensity was used for all other frequencies.

The most effective inhibitory frequency (5–10 Hz) determined under the isovolumetric conditions was tested during repeated CMGs. Each CMG was always started with an empty bladder. The saline infusion (0.5–4 ml/min) was stopped after the onset of the first micturition reflex contraction which had a large amplitude (>30 cmH₂O) and long duration (>30 sec). The infusion rate was kept the same throughout the experiment in each animal. Initially, the CMG was repeated two to three times without stimulation to verify reproducibility and to determine the control bladder capacity. Then, the inhibitory pudendal stimulation was applied during CMGs to evaluate the effect of stimulation on bladder capacity and other urodynamic parameters. At the end of repeated tests of pudendal nerve stimulation, a CMG without stimulation was performed again to verify the reversibility of the inhibitory effects. The bladder was emptied after each CMG followed by a 5–10 minute resting period to allow the bladder reflex to recover.

Naloxone Administration

After determining the effective inhibitory frequencies, the bladder was filled to a volume between 100–110% of the control capacity in order to induce isovolumetric rhythmic bladder contractions. The effective inhibitory stimulation (5–10 Hz) at intensities 1–3 times the inhibition threshold was then applied before and after increasing cumulative doses of naloxone (0.01, 0.03, 0.1, 0.3, and 1 mg/kg, i.v.). Naloxone was administered at 20–30 min intervals. In some instances, naloxone induced a transient increase of isovolumetric bladder activity that usually started 1–2 minutes after naloxone administration and lasted about 5–15 minutes. The

stimulation was always applied after the transient naloxone excitatory effect terminated. After administration of the highest dose of naloxone (1 mg/kg), different stimulation frequencies (1–40 Hz) were tested again to examine the influence of naloxone on the frequency dependent inhibitory pudendal-to-bladder response. The bladder was then emptied and a control CMG in the absence of stimulation was performed to determine the bladder capacity after naloxone treatment. Following the naloxone control CMG, additional CMGs were performed while applying the stimulation to determine the effect of naloxone on pudendal inhibition.

Data analysis

For the isovolumetric bladder contractions, the area under the bladder contraction curve during pudendal nerve stimulation was normalized to the measurement obtained prior to stimulation during the same time period as the stimulation duration (see the inserted tracing in Fig. 1C). In order to detect a long-lasting naloxone effect on isovolumetric bladder contractions, the area under bladder contraction curve, the average peak contraction amplitude, and the baseline bladder pressure after each dose of naloxone were also measured during a 2–5 minute period after recovery from the transient naloxone excitatory effect. These data were normalized to their corresponding measurements during the control period before naloxone administration. For the CMG recordings, bladder capacity, micturition threshold pressure, as well as amplitude, and duration of the first micturition contraction determined from the repeated CMGs were normalized to the measurements of the first control CMG in the same animal so that the results from different animals could be compared. Repeated measurements under the same conditions in the same animal were averaged. The results from different animals were averaged and reported as mean $\pm$ standard error (SE). Statistical significance ($P < 0.05$) was detected with ANOVA and one-sample or paired Student's *t*-test.

RESULTS

Intensity and frequency dependent inhibitory effect of pudendal nerve stimulation

The inhibition of rhythmic bladder contractions under isovolumetric conditions was dependent on the intensity and frequency of pudendal nerve stimulation (Fig. 1). Complete inhibition was elicited in all animals at stimulus intensities between 1 V and 6 V when 5 Hz stimulation was applied. However at low intensities below the threshold for inducing complete inhibition the responses varied. In some experiments ($n=6$) subthreshold stimulation caused incomplete inhibition consisting of a reduction in the amplitude of the contractions or an increase of the inter-contraction intervals. In other experiments ($n=2$) the bladder activity was either not changed or slightly facilitated during stimulation but it was suppressed after the stimulation was terminated (Fig. 1A). The intensity threshold (*T*) for inducing a complete inhibition during 5 Hz stimulation was determined as 2 V in Fig. 1A. Once the stimulation intensity was above this inhibition threshold, complete inhibition was always achieved. Therefore, stimulation intensity of 1–3 *T* was used in this study to test the effect of naloxone treatment.

At intensities above the inhibition threshold determined at 5 Hz stimulation, the effect of pudendal nerve stimulation on isovolumetric bladder contractions was dependent on stimulation frequency (Fig. 1B). Frequencies between 5 Hz and 10 Hz at 1–6 V significantly ($P < 0.05$) suppressed the isovolumetric bladder contractions (Fig. 1C), while stimulation at higher (20–40 Hz) or lower (1–3 Hz) frequencies did not produce inhibition. Facilitation of bladder contractions was observed in some instances during pudendal nerve stimulation at higher (20–40 Hz) or lower (1–3 Hz) frequencies (Fig. 1B), but did not occur consistently across all animals and was not statistically significant (Fig. 1C).

Influence of naloxone on rhythmic bladder activity under isovolumetric conditions

Administration of cumulative doses of naloxone (0.01–1 mg/kg) elicited a transient increase in rhythmic bladder activity under isovolumetric conditions in half of the experiments (Fig. 2). This was evident as an increase in the amplitude and duration of the rhythmic contractions (Fig. 2A1) or as an elevation of the baseline bladder pressure (Fig. 2B1). The transient effect appeared 1–2 minutes after a single low dose of naloxone (0.01–0.1 mg/kg) and lasted about 5–15 minutes. However it did not occur following higher doses (0.1–1 mg/kg, Fig. 2A2 and Fig. 2B2) administered later in the same animals. In some experiments where a transient response occurred, cumulative doses of naloxone did not increase either contraction amplitude or baseline pressure during the time period after the transient response terminated (Fig. 3A). But in other experiments, increasing cumulative doses of naloxone induced a gradual increase in contraction amplitude or baseline pressure (Fig. 3B). This indicates that the transient naloxone response was not necessary for the increase of baseline bladder activity under isovolumetric conditions. On average, the data from all experiments (n=8) revealed that naloxone (0.01–1 mg/kg) did not significantly change the baseline bladder activity under isovolumetric conditions (Fig. 4A–C) except the transient response. The data in Fig. 4A–C were measured during the time period after the transient naloxone response terminated as shown in Fig. 3.

Influence of naloxone on inhibitory pudendal-to-bladder reflex under isovolumetric conditions

The pudendal nerve stimulation was always applied during isovolumetric bladder contractions after the transient naloxone effect terminated. The suppression of isovolumetric bladder contractions induced by pudendal nerve stimulation at threshold intensity for producing complete inhibition was reduced in a graded manner as the dose of naloxone was increased. The results from a single experiment are shown in Fig. 5A. Here a stimulus intensity (4 V) that was just sufficient to completely inhibit the bladder activity in the control period was ineffective after 0.03 mg/kg and 0.1 mg/kg naloxone. However, increasing the stimulation intensity to 5 V restored the inhibition. A higher dose of naloxone (0.3 mg/kg, Fig. 5A) blocked the inhibitory response to 5 V stimulation, but a further increase in stimulus intensity to 6 V restored the inhibition. This inhibition induced by higher stimulus intensity (1.5 times threshold) was resistant to the highest dose of naloxone (1 mg/kg, Fig. 5A). Similar results were obtained in 6 additional experiments in which stimulation of the pudendal nerve at intensities near the inhibition threshold elicited an inhibitory effect that was significantly reduced by a high dose of naloxone (0.3 mg/kg, Fig. 5B), but pudendal nerve stimulation at 1.5–3 times the inhibition threshold could overcome the naloxone effect and significantly ($P<0.05$) suppress the isovolumetric bladder activity (Fig. 5B). After administering the maximal dose of naloxone (1 mg/kg), the inhibition of isovolumetric bladder contractions during pudendal nerve stimulation was still frequency dependent (Fig. 6A), occurring at frequencies between 5 Hz and 10 Hz but not at other frequencies (Fig. 6B).

Influence of naloxone on micturition reflex and pudendal-to-bladder reflex during CMGs

Before administration of naloxone, pudendal nerve stimulation at frequencies (5–10 Hz) and intensities (1T) for completely inhibiting isovolumetric bladder contractions significantly ($P<0.05$) increased bladder capacity to $155.1 \pm 24.5\%$ of control capacity (Fig. 7A–B). Higher intensities of stimulation (1.5–3 T) elicited similar changes (increase to $165.7 \pm 24.9\%$ of control, Fig. 7B). After administration of the maximal dose of naloxone (1 mg/kg) which significantly ($P<0.05$) reduced bladder capacity to $43.0 \pm 11.1\%$ of control, 5–10 Hz pudendal nerve stimulation at 1T intensity completely reversed the facilitatory effect of naloxone ($104.1 \pm 24.5\%$ of the control volume before naloxone) but did not elicit an increase in bladder capacity above the untreated control level (Fig. 7A–B). However higher intensities of nerve stimulation

(1.5–3 T) still significantly ($P<0.05$) increased bladder capacity to $163.4 \pm 10\%$ of the control capacity measured before naloxone treatment (Fig. 7B).

Before naloxone treatment pudendal nerve stimulation significantly ($P<0.05$) increased the micturition threshold pressure, but did not significantly change either the amplitude or the duration of the micturition contraction (Fig. 8A–C). After naloxone treatment which reduced the amplitude and duration of the first micturition contraction (Fig. 7A and Fig. 8B–C) without significantly changing the micturition threshold pressure (Fig. 8A), pudendal nerve stimulation significantly ($P<0.05$) increased the micturition threshold pressure as well as contraction amplitude (Fig. 8A–B).

DISCUSSION

Naloxone, an opioid receptor antagonist, significantly reduced the inhibition of reflex bladder activity induced by low intensity (1 T) pudendal nerve stimulation but did not affect the inhibition induced by high intensity stimulation (1.5–3 T) (Fig. 5 and Fig. 7). Naloxone alone also consistently reduced bladder capacity during CMGs (Fig. 7) and in some experiments induced a transient increase in isovolumetric bladder contractions (Fig. 2) or baseline intravesical pressure (Fig. 3) suggesting that the micturition reflex pathway is under tonic enkephalinergic inhibitory control. These results raise the possibility that the inhibitory pudendal-to-bladder reflex is: (1) dependent in part on release of enkephalins and activation of opioid receptors, (2) facilitated by tonic activation of opioid receptors or (3) suppressed by the bladder hyperreflexia induced by naloxone.

The frequency dependent inhibitory pudendal-to-bladder reflex has been demonstrated in previous studies in both spinal intact (Shen et al., 2008; Woock, 2008) and chronic spinal cord injured cats (Tai et al., 2006, 2008; Wang et al., 2009). This study further revealed that this frequency dependence was not changed by administration of naloxone (Fig. 1 and Fig. 6), indicating that opioid receptors do not play a role in defining the frequency-dependent property of the inhibitory pudendal-to-bladder reflex. Therefore, this property could be determined by other neurotransmitter mechanisms or by the characteristics of the central neural network that processes the pudendal afferent input.

However, this study revealed that opioid receptors might contribute to or modulate the inhibitory pudendal-to-bladder reflex. As the dose of naloxone was increased and more opioid receptors were blocked the inhibition at low intensities of stimulation was reduced (Fig. 5). In order to achieve the same inhibitory effect, higher stimulation intensities were needed suggesting that tonic opioid receptor activation controlled the gain of the inhibitory pathway (Fig. 5). This gain control mechanism was also evidenced by the fact that naloxone decreased the ability of pudendal nerve stimulation to increase bladder capacity at a low stimulation intensity (1 T in Fig. 7B), but not at high stimulation intensity (1.5–3 T in Fig. 7B).

The restoration of inhibition after naloxone administration by increasing the intensity of pudendal nerve stimulation clearly indicates that multiple neurotransmitters must be involved in the inhibition. A previous study (Booth et al., 1985) showed that low doses of naloxone (0.01–0.1 mg/kg, i.v.) could maximally reduce bladder capacity indicating that tonically active opioid receptors in the micturition reflex pathway were already blocked in this dosage range. Therefore, at the maximal dose of naloxone (1 mg/kg) used in this study the majority of opioid receptors involved in micturition reflex must have been blocked (Booth et al., 1985; Hisamitsu and de Groat, 1984; Noto et al., 1991; Roppolo et al., 1983; Thor et al., 1983). However, inhibitory pudendal nerve stimulation at a higher intensity (1.5–3 T in Fig. 5B and Fig. 7B) could still significantly inhibit bladder activity. This indicates that in addition to opioid release pudendal nerve stimulation might also induce the release of other neurotransmitters such as

glycine or GABA. Thus further studies are needed to determine the involvement of other neurotransmitter mechanisms in the inhibitory pudendal-to-bladder reflex.

An analysis of the effect of naloxone on the inhibitory pudendal-to-bladder reflex is complicated due to the facilitatory effect of naloxone on bladder activity. As reported previously (Booth et al., 1985; Dray and Metsch, 1984; Hisamitsu and de Groat, 1984; Noto et al., 1991; Roppolo et al., 1983; Su et al., 1997; Thor et al., 1983) naloxone significantly decreased the bladder capacity (Fig. 7) and also induced transient increases in the isovolumetric bladder activity lasting about 5–15 minutes (Fig. 2). Because bladder hyperactivity might indirectly reduce the effectiveness of inhibitory pathways it is possible that the antagonism of the pudendal inhibition observed during CMGs (1 T in Fig. 7B) could be related to the excitatory effect of naloxone on micturition reflex rather than to a direct suppression of the inhibitory synaptic mechanisms activated by pudendal nerve stimulation. To minimize this complication we tested pudendal nerve stimulation on isovolumetric bladder activity after recovery from the transient facilitatory effect of naloxone, where naloxone did not significantly change the baseline bladder activity (Fig. 4), but it significantly reduced the pudendal inhibition at low (1 T) stimulation intensity (Fig. 5). Based on these experiments it seems reasonable to conclude that naloxone directly suppresses the mechanism underlying pudendal-to-bladder inhibition (Figs. 4–5). However during CMG experiments the facilitatory effect of naloxone on bladder capacity was prolonged and therefore nerve stimulation was tested during the facilitation. Under these conditions naloxone did reduce the increased bladder capacity elicited by pudendal nerve stimulation (Fig. 7) but it is possible that part of this effect of naloxone is due to the enhanced bladder reflex activity which indirectly reduces the efficacy of the inhibitory mechanism.

A previous study in cats (Mazieres et al., 2006) encountered a similar problem in examining the effect of naloxone on recurrent inhibition and somatic afferent nerve inhibition of reflex firing on bladder nerves. In that study naloxone also facilitated the bladder reflexes but the investigators compensated for the facilitation by adjusting stimulus intensities and reducing the magnitude of test reflexes back to control levels. Using this method it was shown that naloxone suppressed recurrent inhibition but did not alter the inhibition induced by stimulation of the dorsal clitoral nerve (a branch of pudendal nerve). These data coupled with the results of the present experiments indicate that multiple afferent pathways in the pudendal nerves can elicit inhibition of bladder reflexes and that inhibition can be mediated by different mechanisms; some dependent and others independent of opioid receptor activation.

It is likely that pudendal nerve stimulation suppresses the micturition reflex by activating inhibitory mechanisms at multiple sites in the central nervous system. Electrophysiological studies in cats revealed that stimulation of afferents in the pudendal nerve directly inhibits firing of bladder preganglionic neurons as well as activity in interneuronal pathways projecting to the preganglionic neurons (de Groat, 1978; de Groat et al., 1982; de Groat and Ryall, 1969). Pudendal nerve stimulation significantly increased the micturition threshold pressure both before and after naloxone treatment (Fig. 8A) indicating that the pudendal afferent input probably inhibited the afferent limb of the spinobulbospinal micturition reflex either at the spinal cord level or at the supraspinal level (Fowler et al., 2008). On the other hand, the efferent limb of the micturition reflex pathway does not seem to be influenced by continuous inhibitory pudendal afferent input before naloxone treatment, because the amplitude and duration of the micturition contractions were not changed by pudendal nerve stimulation (Fig. 8B–C).

Previous studies (Dray and Metsch, 1984; Hisamitsu and de Groat, 1984; Noto et al., 1991) in which naloxone or opioid peptides were applied intrathecally or intracerebroventricularly indicated that opioid receptors in the brain seem to control the frequency of bladder contractions and bladder capacity; whereas those in the spinal cord seem to control the amplitude of the

bladder contractions. Bladder capacity is dependent on a number of factors including: (1) bladder compliance, (2) excitability of bladder mechanosensitive afferents, (3) central processing of bladder afferent input, and (4) sensitivity of the micturition switching circuit in the brain. Naloxone most likely changes bladder capacity by modifying factors 3 and 4. Thus the naloxone-sensitive component of the pudendal-to-bladder inhibitory mechanism might involve activation of inhibitory synapses at spinal or supraspinal levels.

All experiments in this study were conducted under α -chloralose anesthesia that could have a persistent inhibitory effect on the micturition reflex pathway. However, the effects of naloxone (i.e., excitation of bladder and antagonism of pudendal inhibition) are less likely to be dependent on the anesthesia, because naloxone also caused bladder excitation in awake spinal cats (Thor et al. 1983) and in decerebrate unanesthetized cats (Noto et al., 1991). In addition pudendal inhibition was also demonstrated in awake spinal cats (Tai et al., 2008). Our preliminary test (un-published data) also indicated that naloxone could antagonize pudendal inhibition at low stimulation intensity in awake spinal cats. Also, uniphasic stimulus pulses were used in this study instead of biphasic charge-balanced stimulus pulses. It is known that uniphasic pulse can induce electrolysis and potentially damage nerves in chronic application. However, in this study the stimulation was only applied acutely during the experiment. The nerve was not damaged by the stimulation as evidenced by the effectiveness of repeated pudendal nerve stimulation to inhibit reflex bladder activity.

In summary, this study revealed the involvement of opioid receptors in modulating the inhibitory pudendal-to-bladder reflex. However further studies are necessary to examine the involvement of other neurotransmitters such as glycine or GABA in order to fully elucidate the mechanisms underlying the clinical efficacy of pudendal neuromodulation. More information about neurotransmitters might also promote the development of new, more effective treatments combining neuromodulation and pharmacotherapy.

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Fig. 1.

Intensity and frequency dependent inhibitory effect of pudendal nerve stimulation on isovolumetric bladder contractions. A. At low intensities (1.2–1.8 V) of a 5 Hz stimulation, isovolumetric bladder contractions exhibited an initial enhancement of the contraction at the onset of the stimulation followed by a post-stimulus suppression of the contractions. However once the intensity reached the inhibition threshold ($T = 2$ V), the bladder was completely inhibited during the stimulation. B. In another experiment, 5–10 Hz stimulation at inhibition threshold (3 V) completely suppressed contractions and lowered baseline bladder pressure; whereas low (1–3 Hz) and high (20–40 Hz) frequency stimulation elicited a weak excitatory effect that was in some instances (20 Hz) followed by a period of inhibition. C. Summary of

the frequency dependent inhibitory effect induced at stimulation intensity of 1–3 T. The measurement of area under curve during stimulation was normalized to the control measurement before stimulation (see inserted figure in C). Total 8 animals. Stimulation: 1–6 V in C; 0.1 ms pulse width in A–C. * indicates a statistically significant ($P<0.05$) difference from 100%. The thin lines under the pressure traces indicate the zero pressure. The thick black bars under the zero pressure lines indicate periods of stimulation.

Fig. 2.

Changes in bladder activity recorded under isovolumetric conditions after different doses of naloxone (Nx). A. In one experiment a large increase in the amplitude of rhythmic contractions occurred at a dose of 0.01 mg/kg, but not at a higher dosage of 0.1 mg/kg. B. In another experiment, a transient increase in baseline bladder pressure occurred at dose of 0.1 mg/kg, but not at a higher dosage of 1 mg/kg. The thin lines under pressure traces indicate the zero pressure. A2 trace starts about 25 minutes after the end of A1 trace. B2 trace starts about 15 minutes after the end of B1 trace.

Fig. 3. Influence of naloxone (Nx) at different doses on isovolumetric bladder contractions recorded after the transient Nx response (see Fig.2) terminated. A. In one experiment, the baseline bladder pressure and contraction amplitude were not significantly changed. B. In another experiment, both baseline pressure and contraction amplitude were increased. The thin lines under pressure traces indicate the zero pressure.

Fig. 4.

Influence of naloxone (Nx) on isovolumetric bladder contractions during the time period after the transient Nx response terminated (see Fig. 3). A. Normalized area under the bladder contraction curve. B. Normalized peak amplitude of bladder contraction. C. Normalized baseline bladder pressure. Data at different Nx doses were normalized to the measurements during the control periods as indicated in Fig. 3. Total 8 animals.

Fig. 5. Influence of naloxone (Nx) at different cumulative doses (0.01–1 mg/kg) on pudendal inhibition of isovolumetric bladder contractions. A. As the Nx dose increased, the minimal stimulation voltage required to completely inhibit bladder contractions also increased. B. The pudendal nerve evoked inhibitory effect was significantly weakened by Nx at threshold intensity (1 T). However, even after the highest dose of Nx (1 mg/kg) increasing the intensity of stimulation to 1.5–3 T still significantly inhibited bladder activity. Total 7 animals. Stimulation in B: 1–6V, 5–10 Hz. 0.1 ms pulse width in both A and B. * indicates a statistically significant ($P < 0.05$) difference from 100%. # indicates a statistically significant ($P < 0.05$) difference between 1 T and 1.5–3 T groups. The thin lines under the pressure traces in A indicate the zero pressure. The black bars under the zero pressure lines indicate periods of stimulation.

Fig. 6.

Frequency dependent inhibitory effect on isovolumetric bladder contractions after the maximal dose of naloxone (1 mg/kg). A. An example of pudendal nerve inhibitory effect at different frequencies. Black bars under the zero pressure baseline indicate stimulation durations. B. Summary of the frequency dependent inhibitory effect of pudendal nerve stimulation after naloxone treatment. Total 5 animals. Stimulation: intensity 4V for A; 3–6V for B; 0.1 ms pulse width for both A and B. * indicates a statistically significant ($P < 0.05$) difference from 100%.

Fig. 7.

Effect of inhibitory pudendal nerve stimulation on bladder capacity before and after administration of 1 mg/kg naloxone (Nx). A. Repeated CMG recordings with 10 Hz stimulation. Black bars indicate stimulation durations. Th – micturition threshold pressure, A – micturition contraction amplitude, D – micturition contraction duration. B. Summary of the naloxone effect on bladder capacity with 5–10 Hz stimulation. Total 8 animals. T – threshold intensity for completely inhibiting bladder activity under isovolumetric conditions. Stimulation: 4 V (1 T) in A; but 1–6 V in B; 0.1 ms pulse width in both A and B. * indicates statistically significant ($P < 0.05$).

Fig. 8.

Effect of inhibitory pudendal nerve stimulation at frequencies of 5–10 Hz on threshold pressure (A), amplitude (B), and duration (C) of the first micturition contraction during CMG before and after administration of 1 mg/kg naloxone (Nx). The definitions for each measured parameter in A–C were illustrated in Fig. 7A. Stimulation: 1–6 V, 0.1 ms pulse width. *indicates a statistically significant ($P < 0.05$) difference from 100%. Total 8 animals.