Inhibition of bladder overactivity by duloxetine in combination with foot stimulation or WAY-100635 treatment in cats

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Schwen Z, Matsuta Y, Shen B, Wang J, Roppolo JR, de Groat WC, Tai C. Inhibition of bladder overactivity by duloxetine in combination with foot stimulation or WAY-100635 treatment in cats. Am J Physiol Renal Physiol 305: F1663–F1668, 2013. First published October 23, 2013; doi:10.1152/ajprenal.00523.2013.—The purpose of this study was to determine whether duloxetine [a serotonin (5-HT)-norepinephrine reuptake inhibitor] combined with transcutaneous foot stimulation or WAY-100635 (a 5-HT₁A antagonist) can enhance inhibition of bladder overactivity in cats. Cystometrograms were performed on eight cats under α-chloralose anesthesia by infusing saline and then 0.25% acetic acid (AA) to induce bladder overactivity. To inhibit bladder overactivity, foot stimulation (5 Hz) was applied via transcutaneous pad electrodes to the right hindfoot at two and four times the threshold intensity for inducing a toe twitch. Duloxetine (0.003–3 mg/kg) was administered intravenously to determine the effect of combination treatment. After the 3 mg/kg dose of duloxetine, WAY-100635 (0.5 mg/kg) was given intravenously. AA irritation significantly (P < 0.0001) reduced bladder capacity to 42.7 ± 7.4% of the saline control capacity. Foot stimulation alone at both two and four times the threshold intensity significantly (P < 0.0001) inhibited bladder overactivity and increased bladder capacity to 66.7 ± 6.3% and 85.7 ± 6.5% of the saline control, respectively. Duloxetine alone dose dependently inhibited bladder overactivity and completely restored bladder capacity to the saline control (109 ± 15.5%) at 3 mg/kg. Although duloxetine combined with foot stimulation did not further enhance inhibition, WAY-100635 (0.5 mg/kg) given after 3 mg/kg duloxetine further increased (P = 0.008) bladder capacity to 162.2 ± 22.5% of the saline control. Although duloxetine and foot stimulation independently inhibited bladder overactivity, combined treatment did not enhance inhibition. Duloxetine combined with WAY-100635, however, synergistically enhanced bladder inhibition, indicating a potential novel treatment for overactive bladder if duloxetine is combined with a 5-HT₁A receptor antagonist drug.

duloxetine; WAY-100635; bladder; neuromodulation; cat

OVERACTIVE BLADDER (OAB) is a syndrome characterized by urinary urgency, frequency, and incontinence, which occurs in 16–17% of the population and is difficult to manage medically (22). Antimuscarinic drugs remain the first line of therapy, but side effects and relatively low efficacy have resulted in a continued search for alternative treatments for OAB (1, 5). Duloxetine [a serotonin (5-HT)-norepinephrine reuptake inhibitor] that is approved in Europe for the treatment of stress urinary incontinence (24) has also shown efficacy for the treatment of OAB symptoms in multiple animal (35) and clinical studies, including a randomized controlled clinical trial of 306 women (8, 29). However, side effects such as nausea, dry mouth, and dizziness resulted in high patient dropout rates, which appeared to be dose dependent (24).

Neuromodulation therapy (sacral or tibial), which is an alternative to drug treatment, is an effective second line option for refractory OAB (25, 37). However, neuromodulation therapies can be invasive, inconvenient, and costly (25, 37). Our previous studies in cats have revealed a novel, noninvasive method to suppress bladder overactivity by stimulating somatic afferent nerves in the foot with skin surface electrodes. Although foot stimulation alone does not completely suppress bladder overactivity (6, 33), we have recently identified another treatment strategy that combines foot stimulation with low doses of drugs such as tolterodine or tramadol to enhance OAB treatment efficacy while lowering the potential for unwanted side effects (18, 27). The additive effect of neuromodulation and drug therapy has also been demonstrated clinically in patients who had incomplete responses to neuromodulation therapy alone (13). Therefore, in this study, we determined if inhibition of bladder overactivity induced by noninvasive foot stimulation might be augmented by the addition of a low dose of duloxetine that is not effective in inhibiting the bladder. Reducing the dose of duloxetine could minimize the side effects and thus reduce the patient dropout rate. We also investigated whether the effect of duloxetine on bladder overactivity can be augmented when combined with the 5-HT₁A antagonist WAY-100635 to inhibit 5-HT₁A autoreceptors in the raphe nucleus. Previous studies (2, 19) investigating antidepressant effects of 5-HT-norepinephrine reuptake inhibitors have shown that their action on central serotonergic pathways can be amplified when combined with WAY-100635. Because the primary mechanism of action of duloxetine on the bladder is also believed to be through central serotonergic regulation of bladder function (34), we determined if WAY-100635 can enhance the effect of duloxetine on bladder overactivity.

METHODS

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

Experimental setup. Experiments were performed in a total of eight adult anesthetized cats (five female cats and three male cats between 3.1 and 4.4 kg). Each cat was anesthetized with isoflurane (2–3% in O₃) during surgery and then changed to α-chloralose (65 mg/kg, supplemented as necessary) anesthesia during data collection. A pulse oximeter (9847V, Nonin Medical, Plymouth, MN) with the sensor attached to the tongue was used to monitor heart rate and blood oxygen saturation. Catheters were inserted in the right cephalic vein and right carotid artery for the intravenous infusion of drugs and monitoring systemic blood pressure, respectively. Airway access was secured with a tracheostomy tube. Ureters were accessed through a midline incision.
abdominal incision and drained externally. The bladder was then cannulated with a double lumen catheter through a small cut at the proximal urethra to infuse saline or 0.25% acetic acid (AA) and simultaneously measure bladder pressure. The proximal urethra was tied to prevent leakage. Fur was removed from the right hindfoot, and two self-adhesive pad electrodes (diameter: 1 cm, Grass FE10ND, Astro-Medical, Mentor, OH) were attached to the skin at the bottom of the foot. One electrode was at the front of the foot, and the other was at the heel (6, 33).

**Stimulation protocol and drug administration.** Uniphasic rectangular pulses (5-Hz frequency, 0.2-ms pulse width) were delivered to the surface electrodes on the foot. The stimulation intensity threshold was defined as the minimal intensity to induce a toe twitch. Foot stimulation of two and four times the stimulation intensity threshold (2T and 4T, respectively) was used in this study since our previous studies (6, 18, 33) demonstrated that this intensity range was effective in inhibiting reflex bladder contractions.

Initially, the bladder capacity was determined during cystometrograms (CMGs) by slowly infusing the bladder with saline. Multiple CMGs were performed to ensure reproducibility of the saline control capacity. Bladder capacity was defined as the bladder volume threshold required to induce a micturition contraction of large amplitude (>30 cmH₂O) and long duration (>20 s). Repeated CMGs were then performed with an infusion of 0.25% AA to irritate the bladder, activate nociceptive bladder afferent C-fibers, and induce bladder overactivity. When the bladder capacity stabilized, the following four CMGs were performed with AA infusion before the administration of duloxetine: 1) control without stimulation, 2) during 2T stimulation, 3) during 4T stimulation, and 4) control without stimulation to determine any poststimulation effect. The bladder was emptied at the end of each CMG, and a 3- to 5-min rest period was inserted between CMGs. After the predrug CMGs were performed, increasing cumulative doses (0.003, 0.01, 0.03, 0.1, 0.3, 1, and 3 mg/kg iv) of duloxetine (Selleck Chemicals, Houston, TX) were given to determine the drug effect on bladder capacity. Ten minutes after the administration of each dose of duloxetine, the four CMGs were again performed with AA infusion under different conditions (control, 2T stimulation, 4T stimulation, and poststimulation control). The four repeated CMGs were completed within 40–60 min.

In four cats, 50–70 min after the maximal dose of duloxetine (3 mg/kg) was given, WAY-100635 (Sigma-Aldrich, St. Louis, MO), a 5-HT₁₅ antagonist, was administered (0.5 mg/kg iv) to block 5-HT₁₅ inhibitory autoreceptors on serotonergic neurons in the raphe nucleus in an attempt to enhance the serotonergic inhibitory influence of duloxetine on bladder reflexes. Five minutes after the administration of WAY-100635, we performed control CMGs without stimulation to examine the combined effect of WAY-100635 and duloxetine on bladder capacity.

**Data analysis.** For each CMG, bladder capacity was normalized to the initial saline control capacity in the same animal, which allowed for comparisons between animals. Bladder capacities were averaged for each condition and reported with SEs. Student’s t-test, one-way ANOVA followed by Dunnett posttests, or two-way ANOVA followed by Bonferroni posttests was used to determine the statistical significance (P < 0.05).

**RESULTS**

**Suppression of bladder overactivity by foot stimulation.** After saline control CMGs were performed, AA-induced bladder irritation significantly (P < 0.0001) reduced bladder capacity to a mean of 42.7 ± 7.4% (5.7 ± 1.5 ml) of the saline control capacity (11.8 ± 1.8 ml; Fig. 1). Foot stimulation significantly (P < 0.0001) inhibited bladder overactivity and increased the capacity to 66.7 ± 6.3% at 2T and 85.7 ± 6.5% at 4T of the saline control. The poststimulation AA control capacity was not different from the prestimulation AA control, demonstrating that there was no poststimulation effect on bladder capacity (Fig. 1).

**Dose-dependent effect of duloxetine on bladder overactivity with and without stimulation.** In the absence of stimulation, duloxetine dose dependently and significantly (P < 0.05) increased bladder capacity during AA infusion at doses of 0.1–3 mg/kg and completely removed AA-induced overactivity at 3 mg/kg, increasing bladder capacity to 109 ± 15.5% of the saline control (Figs. 2A and 3). When foot stimulation was combined with duloxetine, the stimulation significantly (P < 0.05) increased the capacity after doses ranging from 0.003 to 0.03 mg/kg but did not significantly increase the capacity after doses between 0.1 and 3 mg/kg (Figs. 2 and 3). After the highest dose (3 mg/kg), 2T and 4T stimulation significantly (P < 0.05) increased bladder capacity to 129.3 ± 23.2% and 129.2 ± 25.8% of the saline control, respectively. After 2T and 4T stimulation in duloxetine-treated animals, bladder capacity returned to prestimulation levels, indicating the absence of a poststimulation effect.
Combined effect of duloxetine and WAY-100635. After the final 3 mg/kg dose of duloxetine, intravenous injection of WAY-100635 (0.5 mg/kg), which is a dose that alone does not affect bladder capacity (20, 31, 36), significantly ($P < 0.008$) increased bladder capacity from a mean of 117.3 ± 17.6% to 162.2 ± 22.5% of the saline control (Fig. 4).

DISCUSSION

This study demonstrates that foot stimulation is effective in inhibiting bladder overactivity induced by AA irritation (Fig. 1) and that duloxetine alone can also dose dependently increase bladder capacity, completely reversing the overactivity at the highest dose (3 mg/kg; Figs. 2A and 3). However, the combination of duloxetine and foot stimulation did not exhibit a statistically significant additive benefit at increasing doses of duloxetine. Interestingly, the administration of WAY-100635 (a 5-HT$_{1A}$ antagonist) after the highest dose of duloxetine increased bladder capacity an additional 45% (Fig. 4), whereas the same dose of WAY-100636 alone in previous studies (20, 31, 36) did not increase capacity.

Our results with duloxetine alone on bladder overactivity agree very well with those of a previous study (35) showing a significant increase in bladder capacity in a similar AA-induced bladder overactivity model in cats. That study also demonstrated a return to saline control capacity at 3 mg/kg duloxetine (iv). Our observations also correlate with clinical trials testing duloxetine in OAB patients, which showed a significant improvement in overactive symptoms, including a reduction of daily voids, incontinence episodes, and improvement in quality of life scores (8, 29). Duloxetine, a 5-HT-norepinephrine reuptake inhibitor, is thought to elicit bladder inhibition by facilitating central serotonergic inhibitory pathways. This mechanism is supported by the observation that methiothepin, a nonselective 5-HT receptor antagonist, reversed the effect of duloxetine on bladder capacity (35). Therefore, duloxetine, by increasing extracellular 5-HT levels, may augment the function of bulbospinal serotonergic pathways, which have an overall inhibitory effect on bladder activity (7, 21, 30). In addition, because duloxetine only affects bladder capacity during AA irritation and not during saline irritation, the combination with foot stimulation or WAY-100635 may provide additional benefits.
infusion (34), the inhibition seems to preferentially modulate the nociceptive C-fiber afferent-evoked micturition reflex at the spinal level as opposed to the Aδ-fiber supraspinal micturition pathway, which controls reflex bladder activity under physiological conditions during saline CMGs (10). The inhibition by duloxetine of the nociceptive C-fiber micturition reflex also appears to correlate with its antinociceptive activity in animal models of pain (17, 23) and its clinical efficacy in the treatment of multiple pain syndromes, such as neuropathic pain, fibromyalgia, and osteoarthritis (4, 26, 38). Animal studies (16, 17) have shown that duloxetine can produce analgesia by modulating descending serotonergic and noradrenergic pathways involved in endogenous pain suppression.

Our previous study (32) has shown that opioid receptors play a major role in the inhibition of bladder overactivity by foot stimulation. Tramadol, which has opioid agonist activity, can synergistically enhance foot inhibition and produce a long-lasting poststimulation inhibitory effect (18). However, tramadol also acts as a 5-HT-norepinephrine reuptake inhibitor (15). This raises the possibility that tramadol might also influence foot stimulation by enhancing 5-HT and norepinephrine mechanisms. However, this possibility seems less likely based on our present results showing that duloxetine, a 5-HT-norepinephrine reuptake inhibitor, failed to significantly enhance foot inhibition and did not unmask a poststimulation effect (Fig. 3). This dramatic contrast between the effects of tramadol and duloxetine when combined with foot stimulation suggests that other mechanisms, such as opioid receptor activation, play a major role in foot inhibition of bladder overactivity.

However, even though the effect of foot stimulation on bladder capacity was reduced rather than increased after higher doses (0.1–3 mg/kg) of duloxetine (Fig. 3), this combination experiment where both treatments have an inhibitory effect is complicated and might be open to different interpretations. For example, after doses of duloxetine that significantly increase bladder capacity by enhancing 5-HT levels, it is possible that this action partially occludes a serotonergic mechanism that underlies the effect of foot stimulation. Thus, it is still possible that 5-HT mechanisms may contribute to the inhibition of bladder overactivity by foot stimulation. In addition, duloxetine blocks the reuptake of norepinephrine and enhances noradrenergic synaptic mechanisms, which could also alter the response to foot stimulation. Further studies using selective 5-HT or norepinephrine receptor antagonists are needed to resolve this question.

While duloxetine combined with foot stimulation does not have potential for a clinical application, duloxetine combined with WAY-100635 further increased bladder capacity (Fig. 4). This result suggests that suppressing 5-HT1A receptors can enhance the inhibitory effect of duloxetine on bladder overactivity and might be used to reduce the effective treatment doses of duloxetine to limit both side effects and patient dropout rates. In a large randomized controlled clinical trial investigating duloxetine as a treatment for OAB, 79.1% of treated patients experienced side effects, including nausea (30.7%), dry mouth (16.3%), dizziness (14.4%), and constipation (13.7%), which contributed to a dropout rate of 41% (29). Previous clinical trials of duloxetine demonstrated progressively higher discontinuation rates as the dose increased due to side effects (24). Consequently, lower dropout rates may be achieved by combining a lower dose of duloxetine with a 5-HT1A antagonist drug. While these results are promising, further studies are required to determine the efficacy of low doses of duloxetine in combination with a 5-HT1A antagonist drug for the treatment of bladder overactivity.

It is reasonable to speculate that the additional inhibitory effect on bladder overactivity elicited by WAY-100635 is due to blockade of 5-HT1A autoreceptors expressed by 5-HT neurons in the raphe nucleus, thus enhancing the central serotonergic pathways associated with descending serotonergic mechanisms, which could also alter the response to foot stimulation. Further studies using selective 5-HT or norepinephrine receptor antagonists are needed to resolve this question.

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nergic influence of duloxetine on bladder overactivity. Blockade of these receptors with WAY-100635 removes a negative feedback mechanism and increases the firing of bulbospinal serotonergic neurons (9, 14). In the dorsal raphe nucleus, these 5-HT1A autoreceptors are activated after the administration of 5-HT-norepinephrine reuptake inhibitors, such as duloxetine and venlafaxine, as well as selective 5-HT reuptake inhibitors, resulting in an initial decline in firing due to increased extracellular concentrations of 5-HT caused by reuptake inhibition (2, 3, 11, 12). During continuous administration of 5-HT-norepinephrine reuptake inhibitors, raphe neuron firing remains depressed for months before recovering due to 5-HT1A receptor desensitization (2, 11). Multiple studies (2, 11, 19) have demonstrated that an immediate recovery of raphe neuron firing is elicited in animals treated with 5-HT-norepinephrine reuptake inhibitors. This enhances the central serotonergic drive of duloxetine and likely mediates the enhanced inhibition of bladder overactivity after the administration of WAY-100635 in our experiments. However, other sites of action for the effect of WAY-100635 are also possible because the drug was administered systemically in this study rather than locally in the raphe nucleus.

On the other hand, previous investigations of the functions of 5-HT1A receptors have shown that subcutaneous or intravenous administration of 8-hydroxy-2-di-N-propylaminotetralin (8-OH-DPAT; a 5-HT1A receptor agonist) inhibits bladder activity; this inhibition was reversed by the administration of WAY-100635 (31, 36). However, when WAY-100635 was administered alone, it did not affect bladder overactivity, indicating that 5-HT1A receptors are not tonically active in the micturition reflex pathway (20, 31, 36). 8-OH-DPAT suppresses the micturition reflex in chronic spinal cats (31), indicating that the inhibitory effect is mediated by 5-HT1A receptors located in the spinal cord.

Our recent study (28) has shown that the repeated CMGs as performed in this study are very stable and reproducible. The bladder capacities measured during these repeated CMGs were not changed over seven dosages of vehicle (saline) intravenous injections during a several hour-long experiment (28). Therefore, the effects observed in this study are unlikely to be influenced by variations in bladder capacity during the repeated CMG protocol.

This study demonstrates that 5-HT-norepinephrine mechanisms might play a very limited role in foot inhibition of bladder overactivity, which contrasts with the prominent involvement of opioid mechanisms (6, 18, 33). When duloxetine was combined with the 5-HT1A antagonist WAY-100635, inhibition of bladder overactivity was further enhanced, which reveals a promising synergistic mechanism that could be used for a novel treatment for OAB. Understanding the neurotransmitter mechanisms underlying bladder overactivity and neuromodulation is important for developing new therapies for OAB.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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