Activation of the external urethral sphincter central pattern generator by a 5-HT$_{1A}$ receptor agonist in rats with chronic spinal cord injury

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Dolber PC, Gu B, Zhang X, Fraser MO, Thor KB, Reiter JP. Activation of the external urethral sphincter central pattern generator by a 5-HT$_{1A}$ receptor agonist in rats with chronic spinal cord injury. Am J Physiol Regul Integr Comp Physiol 292: R1699–R1706, 2007. First published January 4, 2007; doi:10.1152/ajpregu.00142.2006.—We recently demonstrated that treatment with the 5-HT$_{1A}$/7 receptor agonist [(R)-(−)-8-hydroxy-2-di-n-propylamino]tetralin (8-OH-DPAT) increases bladder capacity in chloralose-anesthetized female cats with chronic spinal cord injury. In the current study, we investigated the effects of 8-OH-DPAT on bladder capacity and external urethral sphincter (EUS) activity in urethane-anesthetized female rats (initial body mass 175–200 g) with chronic spinal cord injury (transsection at T10). Cystometric study took place 8–12 wk posttranssection. Intra vesical pressure was monitored in urethane-anesthetized rats with a transvesical catheter, and EUS activity was assessed electromyographically. Spinal cord injury disrupts phasic activity of the EUS, resulting in decreased voiding efficiency and increased residual volume. 8-OH-DPAT induced a dose-dependent decrease in bladder capacity (the opposite of its effect in chronic spinal cord-injured cats) with an increase in micturition volume and decrease in residual volume resulting from improvement in voiding efficiency. The unexpected improvement in voiding efficiency can be explained by the 8-OH-DPAT-induced emergence of phasic EUS relaxation. Phasic EUS relaxation was also altered by 8-OH-DPAT in spinally intact rats, whereas the 5-HT$_{1A}$ receptor antagonist N-tert-butyl-3-[4-(2-methoxyphenyl)-piperazin-1-yl]-2-phenylpropamidine (WAY-100635), on its own, was without effect. It remains to be determined when phasic relaxation is restored after spinal cord injury, and indeed whether it is ever truly lost or is only temporarily separated from excitatory input.

bladder; micturition; neurogenic voiding; detrusor; urethra

LIGANDS ACTING AT 5-HT$_{1A}$ receptors modify lower urinary tract function (8, 28). Thus, in spinally intact rats, 5-HT$_{1A}$ receptor agonists increase and 5-HT$_{1A}$ receptor antagonists decrease urinary frequency through actions at both supraspinal and spinal levels (17, 21, 33, 34). The source of most spinal cord 5-HT-containing terminals are neuronal somata located in brain stem raphe nuclei (2). Spinal cord neuronal 5-HT$_{1A}$ receptors, and likewise many 5-HT-containing terminals, are found both in the dorsal horn and in the ventral horn motor nucleus driving the external urethral sphincter (EUS) and external anal sphincter (36). (The ventral horn motor nucleus is known as Onuf’s nucleus in the human; its homologs in the rat are the dorsomedial and dorsolateral nuclei, lumped together as Onuf’s nucleus in this paper, for generality.)

This important supraspinal control system is lost when spinal cord transsection interrupts axons of the brain stem 5-HT-producing neurons. However, 5-HT$_{1A}$ receptors remain within the spinal cord following destruction of descending 5-HT-containing pathways in the rat (20, 29) and spinal cord transsection in the cat (12). Thus, 5-HT$_{1A}$ receptor agonists remain potential therapeutic agents in the setting of spinal cord injury. This therapeutic potential is supported by our recent finding that the 5-HT$_{1A}$/7 receptor agonist [(R)-(−)-8-hydroxy-2-di-n-propylamino]tetralin (8-OH-DPAT) increases bladder capacity in chronic spinal cord-injured cats (15). In contrast, previous studies in rats with chronic spinal cord injury showed that intrathecal 8-OH-DPAT promoted bladder contractions under isovolumetric conditions (21). However, those conditions do not allow for a full assessment of the effects of 5-HT$_{1A}$ receptor ligands on micturition, a process involving the entire lower urinary tract (e.g., bladder capacity, micturition volume, residual volume, and EUS effects).

In spinally intact rats, EUS activity is minimal during voiding and under nociceptive but not innocuous conditions 8-OH-DPAT enhances EUS activity between voids without interfering with the EUS relaxation during voids (35). In spinally intact rats, on the other hand, alternating EUS activation and relaxation occurs at about 6 Hz during voiding (19). Voiding efficiency in spinally intact rats is reduced when this phasic EUS activity during voiding is prevented (37). In spinal cord-injured rats, tonic rather than phasic EUS-electromyography (EUS-EMG) activity occurs during bladder contractions (19), commonly producing overflow incontinence. The effect of 8-OH-DPAT on phasic EUS-EMG activity in spinally intact and spinal cord-injured rats has not been previously described.

In this study, we examine the effect of 8-OH-DPAT on micturition during open cystometry in chronic spinal cord-injured rats, paying particular attention to the activity of the EUS.

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MATERIALS AND METHODS

A total of 20 female Sprague-Dawley rats (Charles River, Wilmington, DE) with an initial body mass of 175–200 g was used. The experimental protocol was approved by the Animal Care and Use Committee of the Durham Veterans Affairs Medical Center. Spinal cord injury was produced in nine of the rats by transection at the T10 level; cystometric study occurred 8–12 wk posttranssection.

Cystometric methods. General cystometric methods were much as we have previously described (14). Rats were anesthetized with urethane (1.3 g/kg sc; Sigma, St. Louis, MO); at this dose level, anesthesia was very long lasting and did not require supplementation. A polyethylene (PE)-50 catheter was placed in the left jugular vein for intravenous drug administration. The urinary bladder was exposed via a midline abdominal incision, a PE-90 catheter with a flared end was inserted through the bladder dome, and a suture was tightened around it. The other end of the bladder catheter was connected to a syringe pump (model 975; Harvard Apparatus, South Natick, MA) for continuous infusion of saline and to a pressure transducer (model P23XL-1; Gould Ohmeda, Valley View, OH) for intravesical pressure monitoring. Physiological saline was infused at room temperature into the bladder to elicit repeated voiding responses. The infusion rate was 0.11–0.15 ml/min for spinally intact rats and 0.30–1.10 ml/min for spinal cord-injured rats (similar to Ref. 19); the higher rate of infusion is necessary in spinal cord-injured rats with their enlarged bladders to ensure the occurrence of a voiding contraction within 10 min of drug administration. The voided fluid was collected in a syringe connected to a force transducer (model A-934; Kulite, Leonia, NJ) which was calibrated to determine the micturition volume. Intravesical pressure and micturition volume were sampled at 50 Hz. The cystometric variables analyzed were micturition volume (voided volume), residual volume (volume remaining in the bladder after voiding), bladder capacity (micturition volume plus residual volume), and peak intravesical pressure.

EUS-EMG. To record the EUS-EMG, 0.005-in. diameter Medwire AGST polytetrafluorethylene-coated silver wire electrodes (Sigmund Cohn, Mount Vernon, NY) were inserted percutaneously into the vicinity of the EUS. The electrodes were connected through a model HIP5 high-impedance probe (Grass, Quincy, MA) to a Grass P5 preamplifier. EUS-EMG data were sampled at 5,000 Hz, 5–10 times the frequency of the major peaks as recommended to prevent aliasing (6). During voiding, the EUS-EMG of urethane-anesthetized spinally intact rats is characterized by a series of alternating bursts and pauses. In urethane-anesthetized spinal cord-injured rats, activity was tonic during voiding contractions (bladder-sphincter dyssynergia), which consequently result only in overflow incontinence; however, pauses interrupting the otherwise tonic activity were elicited by 8-OH-DPAT to consequently result only in overflow incontinence; however, pauses interrupting the otherwise tonic activity were elicited by 8-OH-DPAT to


Table 1. Mean values for cystometric variables during vehicle infusion

<table>
<thead>
<tr>
<th>State</th>
<th>n</th>
<th>MV, ml</th>
<th>RV, ml</th>
<th>Cap, ml</th>
<th>Void Eff, %</th>
<th>No. Pauses</th>
<th>Peak p, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact</td>
<td>5</td>
<td>0.27 (0.11)</td>
<td>0.04 (0.06)</td>
<td>0.31 (0.15)</td>
<td>89.2 (15.0)</td>
<td>14.8 (8.5)</td>
<td>20.0 (8.4)</td>
</tr>
<tr>
<td>SCI</td>
<td>6</td>
<td>0.14 (0.08)</td>
<td>3.68 (1.36)</td>
<td>3.82 (1.40)</td>
<td>4.0 (2.7)</td>
<td>2.0 (2.8)</td>
<td>33.2 (8.3)</td>
</tr>
</tbody>
</table>

Values are means (SD) for 5 intact and 6 SCI rats. SCI, spinal cord injury; MV, micturition volume; RV, residual volume; Cap, bladder capacity (MV + RV); Void Eff, voiding efficiency; No. Pauses, number of external urethral sphincter-electromyography pauses during voiding; Peak p, peak intravesical pressure.
meaningless. However, vehicle averages are given with conventional estimates of mean (SD) in Table 1.

RESULTS

Spinally intact rats and chronic spinal cord-injured rats exhibit very different types of EUS-EMG activity during bladder contractions under urethane anesthesia; spinally intact rats show heightened tonic activity interrupted by phasic relaxations (Fig. 1A), whereas chronic spinal cord-injured rats show only the heightened tonic activity and thus bladder-sphincter dyssynergia (Fig. 1B). In protocol 1, intravenous administration of 8-OH-DPAT to spinal cord-injured rats resulted in dose-dependent emergence of phasic EUS relaxations (Fig. 2A). This drug-induced change was of relatively short duration (in this rat, about 20 min at 0.1 mg/kg iv), reflecting the short half-life of 8-OH-DPAT in vivo.

Because of the short half-life of 8-OH-DPAT action, we used protocol 2 so that each dose of 8-OH-DPAT was given immediately before a single filling cystometrogram was done. The results of such an experiment in one chronic spinal cord-injured rat are shown in Fig. 2B. It is evident that 8-OH-DPAT causes the dose-dependent interpolation of pauses into the otherwise tonic EUS-EMG activity, yielding phasic activity. Although both pauses and bursts appear to be lengthened compared with those of spinally intact rats (vide infra), the basic pattern is very much the same.

Dose-response curves for cystometric variables in spinal cord-injured rats are shown in Fig. 3. With increasing doses of 8-OH-DPAT, micturition volume increased from 0.14 ml (SD 0.08) with vehicle to 0.31 ml (SD 0.14) at 1 mg/kg, whereas decreases were seen for residual volume [from 3.68 ml (SD 1.36) to 2.00 ml (SD 0.64)], bladder capacity [from 3.82 ml (SD 1.40) to 2.31 ml (SD 0.67)], and peak intravesical pressure [from 33.2 mmHg (SD 8.3) to 24.8 mmHg (SD 3.0)]. All of these changes were reversed by treatment with the 5-HT1A receptor antagonist WAY-100635 at 0.3 mg/kg iv (Fig. 4). The rise in micturition volume and fall in bladder capacity concomitant with 8-OH-DPAT treatment indicate an improvement in voiding efficiency, taken as the ratio of micturition volume to bladder capacity and expressed as a percentage, from 4.0% (SD 2.7) with vehicle administration to 14.1% (SD 6.7) with 1 mg/kg 8-OH-DPAT, and back to 4.8% (SD 2.7) following 0.3 mg/kg WAY-100635 (Fig. 4). The changes in micturition volume, residual volume, bladder capacity and voiding efficiency all achieved statistical significance between 0.03 and 0.1 mg/kg (Fig. 3). As shown in the Fig. 3, bottom, right, this is also the point at which the number of pauses interpolated into the EUS-EMG rose dramatically from 2.0/micturition (SD 2.8) with vehicle to 18.2/micturition (SD 3.4) at 1 mg/kg. The increase in number of pauses per micturition was also completely reversed with WAY-100635 (Fig. 4).

We next investigated spinally intact rats. The effects on the EUS-EMG were discernible in some (but not all) rats, as shown in Fig. 5. Figure 5A shows the results from a rat that showed very little change in the duration during which phasic relaxation occurred, but nonetheless showed substantial changes in the lengths of the individual pauses and thus in the number of pauses per micturition contraction. Figure 5B shows the results from another rat that did show a substantial change in the duration during which phasic relaxation occurred and again showed substantial change in the lengths of the individual pauses. Other rats showed no easily discernible changes in EUS-EMG activity. Clearly, more extensive study is needed to fully characterize 8-OH-DPAT effects in spinally intact rats. Associated with the variable changes in EUS-EMG activity,

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**Fig. 1.** The external urethral sphincter-electromyography (EUS-EMG) in spinally intact and spinal cord-injured urethane-anesthetized rats. A: in this spinally intact rat with a filling rate of 0.15 ml/min, the micturition contraction (*) occurred at ~4 min and was associated with markedly heightened EUS-EMG activity; as shown in the 8-s expanded view (right), tonic activity rose during the micturition contraction until phasic activity and intravesical pressure (IVP) high-frequency oscillations occurred (*). B: in this spinal cord-injured rat with a filling rate of 0.42 ml/min, the micturition contraction at about 8 min (*) was preceded by several nonvoiding contractions associated with elevated EUS-EMG activity (2-headed arrow); as shown in the 8-s expanded view (right), the micturition contraction was associated with only tonic and not phasic EUS-EMG activity.
the changes in cystometric variables were not in any case significant (except intermittently for micturition volume) as summarized in Fig. 6. However, there are noteworthy differences that might become statistically significant with larger numbers of animals, especially the number of pauses per micturition (which was significantly increased for 2 of the 3 highest doses without Bonferroni correction).

That 8-OH-DPAT induced phasic relaxation in chronic spinal cord-injured rats suggested the possibility that phasic relaxation depends upon descending 5-HT stimulation of 5-HT1A receptors in the spinally intact rat, which is lost upon spinal cord transection. However, treatment with 0.3 mg/kg WAY-100635 in the six spinally intact rats following the highest dose of 8-OH-DPAT did not suppress phasic relaxation as it did in chronic spinal cord-injured rats. Furthermore, experiments of protocol 3 showed that the number and rate of pauses per micturition were not altered by WAY-100635 treatment (data not shown). Thus, stimulation of 5-HT1A receptors is sufficient but not necessary for the generation of phasic EUS pauses.

**DISCUSSION**

The principal findings of this study are that the 5-HT1A/7 receptor agonist 8-OH-DPAT induces periodic EUS relaxation...
during voiding in urethane-anesthetized chronic spinal cord-injured rats and that this results in an increase in micturition volume, a decrease in bladder capacity, and thus an increase in voiding efficiency, and that these actions are reversed by the 5-HT$_{1A}$ receptor-selective antagonist WAY-100635. These effects are likely brought about both by periodically reducing outlet resistance and by producing a “pumping” action. Additionally, 8-OH-DPAT induces subtler changes in EUS-EMG activity in spinally intact rats, which do not significantly affect cystometric variables, while WAY-100635 is without effect on phasic EUS activity in spinally intact rats [although it is known to affect some other cystometric variables (7, 17)]. Below, we consider these points, in turn, and relate them to schematics of pathways controlling micturition in spinally intact and chronic spinal cord-injured rats depicted in Fig. 7. For convenience, many connections, which might, in fact, be polysynaptic, are shown as monosynaptic, and all elements are depicted as if in the same segment(s), although recent data suggest this is not true for the control of phasic EUS activity (3).

As the bladders of spinally intact rats begin to contract, tonic EUS activity increases before voiding begins (14). This is due mostly to a spinal reflex, given that it survives spinal cord injury even acutely in rats (27, 38) and humans (18, 24), and in that setting is presumably the basis for simultaneous contractions of bladder and EUS (bladder-sphincter dyssynergia). The pathway controlling this spinal tonic EUS reflex is schematized in Fig. 7 as running from afferent neurons to excitatory interneurons to Onuf’s nucleus motor neurons and thence to the EUS. Whereas the EUS is continuously relaxed during voiding in many species including humans, it is phasically relaxed in rats. This requires central pattern generation “in

Fig. 4. The effect of the 5-HT$_{1A}$ receptor antagonist WAY-100635 (WAY; 0.3 mg/kg iv) on responses produced by the maximal dose of 8-OH-DPAT (maxDPAT; 1 mg/kg iv). The WAY-100635 treatment reversed 8-OH-DPAT effects on micturition volume, residual volume, bladder capacity, voiding efficiency, and EUS-EMG pauses per micturition as indicated by the significant differences for those variables between 8-OH-DPAT and WAY-100635, and the lack of significant differences between vehicle and WAY-100635.

Fig. 5. Effect of increasing doses of 8-OH-DPAT on EUS-EMG activity in two spinally intact rats. A: in this rat with numerous EUS-EMG pauses per micturition, the duration of phasic activity was only slightly altered, but the length of individual pauses increased, whereas the number of pauses declined.

B: in this rat with few EUS-EMG pauses per micturition, both the duration of phasic activity and the length of individual pauses increased. Some rats showed no obvious difference in duration of phasic activity or of pauses.
which oscillatory motor output is generated in the absence of any oscillatory input" (11). Whereas central pattern generators typically involve the generation of bursts of activity against a silent background, the central EUS pattern generator may be a "pause generator" that interpolates pauses into the otherwise tonic activity arising from the spinal tonic EUS reflex (14). We have schematized this in Fig. 7, top as being due to DGC inhibitory interneurons, which exhibit self inhibition (31) such that their activation quickly leads to their inhibition, which again permits their excitation, and so on. The self-inhibition may actually depend upon polysynaptic rather than monosynaptic connections; for example, the same effect would be achieved if the DGC interneurons inhibit those neurons responsible for their excitation. This interpretation leads to the recognition that micturition in humans and rats is more similar than is commonly believed; the rat simply has periodic rather than tonic relaxation during voiding.

Complete spinal cord injury generally prevents tonic relaxation of the EUS in humans during voiding contractions (1, 30) and similarly prevents phasic relaxation of the EUS in deeply urethane-anesthetized spinal cord-injured rats. This markedly decreases voiding efficiency, which can be improved in both humans (9, 22) and rats (37) by enforcing EUS relaxation with neuromuscular blockade. The present study shows that 8-OH-DPAT administration restores phasic relaxation of the EUS in urethane-anesthetized chronic spinal cord-injured rats and by this quite different mechanism increases voiding efficiency.

In spinally intact rats, in which phasic EUS relaxation is already effective in appropriately lowering urethral resistance during bladder contractions, 8-OH-DPAT correspondingly had very little effect on cystometric variables. This is not surprising, given that increasing the duration of individual phasic relaxations might either antagonize or promote efficient voiding in spinally intact rats. Thus, in those spinally intact rats that had numerous phasic relaxations before drug treatment, the lengthening of relaxations could actually antagonize efficient voiding, given that complete blockade of EUS contractions in spinally intact rats with α-bungarotoxin reduces voiding efficiency (37). On the other hand, in spinally intact rats with infrequent phasic relaxations before drug treatment, voiding efficiency could presumably be enhanced. These speculations must be conditioned by at least two other crucial considerations: 1) augmented phasic relaxation outliving the length of the bladder contraction, assuming that is possible, cannot improve voiding efficiency; and 2) voiding efficiency cannot be improved beyond 100% (which it regularly approaches or attains in spinally intact rats; see Fig. 6).

In any case, that 8-OH-DPAT modified EUS-EMG pause generation both in some spinally intact rats and in chronic spinal cord-injured rats suggests that the same pattern generator is being targeted in both spinally intact and chronic spinal cord-injured rats and that in both cases the central EUS pattern generator has a spinal locus rather than a supraspinal one. The mechanism underlying the rat’s central EUS pattern generator is unknown. However, while 8-OH-DPAT can induce pauses in urethane-anesthetized chronic spinal cord-injured rats, 5-HT input cannot be a requirement for pause generation in conscious rats. First, as shown in this study, WAY-100635 does not affect pause generation in spinally intact rats. Second, pauses resume in conscious rats with chronic complete midthoracic spinal cord transection (4) in which descending 5-HT input has been irreversibly removed (23, 26).

Sustained excitatory input to the DGC inhibitory interneurons during the micturition contraction is a crucial part of the generation of phasic activity. Since this input is lost upon transection, plasticity in the form of replacement of lost su-
praspinal inputs with new inputs, perhaps from the same afferent neurons driving the spinal tonic EUS reflex, would have to develop. Similar problems beset other hypothetical mechanisms. Thus, even if the elements of the spinal EUS pattern generator remain (explaining the conserved pattern of phasic relaxation in chronic spinal cord-injured rats), spinal cord plasticity must occur for activation of the spinal pattern generator. The locus and even the timing of such plasticity remain unknown, which is schematically represented in Fig. 7, bottom, as being due to spinal micturition reflex neurons, which could be projection neurons, excitatory interneurons innervating Onuf’s nucleus, or other interneurons.

It must be noted that activation of bladder contraction, and subsequently of pause generation, may require a switch mechanism just as in spinally intact rats. In spinally intact rats, the switch is located in the pons (Fig. 7, top); excitatory output to the SPN parasympathetic preganglionic neurons (and to the spinal EUS pattern generator) occurs when the switch is closed, leading to the sudden generation of high bladder pressures from a low threshold value. The rapid rise and fall in bladder pressure, which is the ultimate sign of switch activity (just as sudden pontine neuronal output is the proximate sign), also occurs in chronic spinal cord-injured rats whether open or isovolumic cystometry is employed. Certainly, the rapid rise in bladder pressure indicates that SPN parasympathetic preganglionic neuronal output is not the consequence of a gradual monosynaptic increase in afferent input. Conceivably there could be a switch-like increase of afferent input due to the sudden activation of a set of previously silent afferent neurons, which would have to be capsaicin-insensitive fibers, because the spinal micturition reflex in the chronic spinal cord-injured rat survives capsaicin treatment (5). In any case, we suggest that the switch in chronic spinal cord-injured rats has a spinal locus and that its activation by afferent neurons drives the SPN parasympathetic preganglionic neurons.

Our hypothetical framework for coordinated bladder contraction and phasic EUS relaxation in the chronic spinal cord-injured rat thus includes a capsaicin-insensitive afferent input to a switch, which, just as in the intact rat, directly or indirectly controls excitation of the parasympathetic preganglionic neurons. Because phasic EUS relaxation is not seen except in the setting of a bladder contraction, we assume that activation of the switch is required for activation of the spinal EUS pattern generator. Because spinal micturition reflex-driven voiding contractions occur in tandem...
with phasic relaxation (this study and Ref. 4), the switch must be found proximal to both SPN parasympathetic preganglionic neurons and the spinal EUS pattern generator (Fig. 7, bottom). We have schematized the limb of the reflex pathway, which activates the EUS pattern generator to emphasize three important features. It is inhibited by urethane, because phasic relaxation occurs in chronic spinal cord-injured rats that are conscious (4) but not in those that are urethane-anesthetized (e.g., pre-8-OH-DPAT rats in this study). It is stimulated by 8-OH-DPAT (this study). It is apparently either absent or inhibited in humans, for which reason humans maintain bladder-sphincter dysynergia. It may additionally be inhibited by κ₁ opiate receptor agonists as it is in spinally intact rats (14). It remains to be demonstrated when the EUS pattern generator is restored after spinal cord injury, whether it is ever truly lost as opposed to merely being separated from its excitatory input, and finally whether the human analog (which yields tonic rather than phasic relaxation) can be activated in humans after spinal cord injury.

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