Inhibition of Bladder Activity by 5-Hydroxytryptamine$_1$ Serotonin Receptor Agonists in Cats with Chronic Spinal Cord Injury

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ABSTRACT

The serotonin (5-hydroxytryptamine$_1$) 5-HT$_{1A}$ receptor agonist 8-OH-DPAT ([(R)-(-)-8-hydroxy-2-[dimethylamino]tetralin]) inhibits bladder activity under nociceptive but not innocuous conditions in cats with an intact spinal cord, suggestive of an effect on primary afferent C fibers or their targets. Because C fibers play a key role in reflex micturition in chronic spinal cord injury (SCI), we investigated the effect of 8-OH-DPAT on micturition in SCI cats. We also investigated GR-46611 (3-[3-(2-dimethylaminooethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl)acrylamide), which has agonist activity predominantly at 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors but also at the 5-HT$_{1A}$ receptor. Chloralose-anesthetized cats were catheterized through the bladder dome for saline-filling cystometry. Dose-response curves for i.v. 8-OH-DPAT (0.3–30 μg/kg) and GR-46611 (0.03–300 μg/kg) were followed in three cases each by 5-HT$_{1A}$ antagonist WAY-100635 [N-tert-butyl-3-(4-[2-methoxyphenyl]-piperazin-1-yl)-2-phenylpropanamide] at 300 μg/kg. Threshold volume, capacity, residual volume, micturition volume, and arterial pressure were measured. Intact cats showed few significant changes in cystometric variables. SCI cats responded to both 8-OH-DPAT and GR-46611 with dose-dependent increases in threshold volume, capacity, and residual volume, significant at ≥10 μg/kg for 8-OH-DPAT and at ≥3 μg/kg for GR-46611. Effects of 8-OH-DPAT but not GR-46611 were largely reversed by WAY-100635. Both 5-HT$_{1A}$ and 5-HT$_{1B/1D}$ agonists may offer a promising means of reducing bladder hyperactivity and increasing bladder capacity in patients with chronic SCI.

With spinal cord injury (SCI) rostral to the lumbosacral level, the most common constellation of lower urinary tract symptoms is loss of voluntary control of micturition, bladder hyperactivity mediated by spinal reflex pathways, and hyperreflexia or spasticity of the external urethral sphincter (EUS), with bladder and EUS activity commonly being dys-synchronous (bladder-sphincter dyssynergia) (Arnold, 1999; Madersbacher et al., 1999; Yoshimura et al., 2000). The patient is thus left in a state of unbalanced reflex incontinence, for which the management of choice is currently intermittent self-catheterization (Madersbacher et al., 1999). Pharmacotherapy that reduces bladder hyperactivity, improves EUS function, and increases functional bladder capacity could be beneficial in maintaining continence between catheterization, reducing kidney damage that results from high intravesical pressure, and reducing autonomic dysreflexia associated with bladder hyperreflexia.

Primary afferent C fibers and the second order neurons with which they communicate are attractive targets for pharmacotherapy. Whereas mechanoreceptive Aδ fibers participate in the normal spinobulbospinal micturition reflex, C fibers are generally silent during bladder filling in normal animals and are activated only in nociceptive conditions (de Groat et al., 1990). In chronic SCI, the spinobulbospinal reflex is lost and C fibers participate in the purely spinal micturition reflex (de Groat et al., 1990). Pharmacotherapy aimed at bladder nociceptors and the neurons integrating their input is thus likely to be useful.

Serotonin 5-HT$_{1}$ receptor agonists are potentially useful therapeutic compounds. Serotonin is antinociceptive (Millan, 1997), perhaps largely through actions at 5-HT$_{1A}$, 5-HT$_{1B}$, and 5-HT$_{1D}$ receptors, all of which are abundant in the dorsal horn of the spinal cord (Thor et al., 1993; Millan, 1997). Most

ABBREVIATIONS. SCI, spinal cord injury; EUS, external urethral sphincter; 5-HT, 5-hydroxytryptamine; EMG, electromyogram; 8-OH-DPAT, (R)-(-)-8-hydroxy-2-[dimethylamino]tetralin; GR-46611, 3-[3-(2-dimethylaminooethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl)acrylamide; WAY-100635, N-tert-butyl-3-(4-[2-methoxyphenyl]-piperazin-1-yl)-2-phenylpropanamide.
spinal serotonergic terminals originate from bulbospinal midbrain raphe neurons, and spinal cord injury results in the loss of these terminals. However, the 5-HT$_1A$ receptors for serotonin persist in the dorsal horn of animals with chronic SCI (Giroux et al., 1999).

In a recent cystometric study of the 5-HT$_1A$ receptor agonist (R)-(+)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) (Thor et al., 2002), normal cat bladders were filled either with saline to innocuously initiate micturition via activation of myelinated A$_\delta$ fibers or with dilute acetic acid to nociceptively initiate micturition via activation of unmyelinated C fibers. During innocuous saline infusion, 8-OH-DPAT treatment did not significantly alter bladder activity or the EUS-EMG. During nociceptive acetic acid infusion, however, 8-OH-DPAT markedly inhibited bladder activity and increased EUS-EMG activity. Both of these effects could promote urine storage, by relaxing the bladder and at the same time facilitating closure of the EUS. The 5-HT$_1A$ antagonist WAY-100635 had no effect in the absence of 8-OH-DPAT but reversed all the effects of 8-OH-DPAT. The reversal with WAY-100635 shows that the effects of 8-OH-DPAT were mediated by 5-HT$_1A$ rather than 5-HT$_3$ receptors, to which 8-OH-DPAT also binds (Hoyer et al., 1994). 8-OH-DPAT had no facilitatory effects on EUS reflexes evoked by low-threshold electrical stimulation of non-nociceptive afferent fibers (Thor et al., 2002), supporting the hypothesis that 5-HT$_1A$ agonist actions were mediated along the nociceptive afferent pathway as opposed to directly on the EUS motor neurons.

These effects of 8-OH-DPAT treatment appear to be due to stimulation of 5-HT$_1A$ receptors on nociceptive C fibers or associated second-order neurons, since they played a significant role only with nociceptive infusate. Because C fibers play an important role in bladder function in the SCI cat (de Groat et al., 1990), 5-HT$_1A$ receptor agonists may provide a therapeutic target for control of bladder function in SCI cats.

Recent studies have also shown that agonists at 5-HT$_1B$ and 5-HT$_1D$ receptors, which are also plentiful in the dorsal horn, are also effective in reducing bladder activity initiated by acetic acid infusion (K. B. Thor, M. A. Katofiasc, J. Schaus, and D. L. Nelson, unpublished observation). Thus, the response to a 5-HT$_1B/1D$ receptor agonist was also tested in the current studies to provide evidence that this class of agent might also benefit patients with SCI by suppressing bladder hyperreflexia and facilitating urine storage.

**Materials and Methods**

**Animals.** A total of 18 female cats (Harlan, Indianapolis, IN) weighing 2.1 to 3.8 kg and aged 0.6 to 1.5 years were used. The experimental protocol was approved by the animal care and use committee of the Durham Veterans Affairs Medical Center. These studies were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the U.S. National Institutes of Health.

**Spinal Cord Injury Model (13 Cats).** Anesthesia was induced with ketamine (100 mg i.m.) and maintained with halothane. A laminectomy was then performed at the T12-T13 vertebral level. The dura mater and spinal cord were cut to produce a 3- to 4-mm gap between the cut ends, and a piece of Gelfoam was inserted in the gap. The muscle and skin were then sutured, and the animal was returned to its cage. Buprenorphine (0.1 mg/kg i.m.) was used for analgesia immediately postoperatively.

The cats consistently did very well after SCI surgery. They received prophylactic ampicillin treatment (150 mg/kg i.m.) beginning on the day of surgery and continuing for 3 days. Within 3 days postoperatively, they were ambulatory using their front legs, ate well, appeared behaviorally well adjusted, did not evince signs of pain or depression, and did not engage in autotomy. The bladders of all cats were manually expressed twice a day after spinal cord transaction surgery, and the volume of urine expressed was recorded.

Survival after spinal cord transaction was 8 weeks to ensure that SCI-induced changes in the distribution of 5-HT$_1A$ receptors became stable (Giroux et al., 1999) and to allow time for development of the spinal C fiber micturition reflex, which normally emerges at about 1 to 2 weeks post-transection (de Groat et al., 1981, 1997; Yoshimura, 1999). In our experiments, emergence of this reflex is evidenced cystometrically (except in one case shown in Fig. 5) by the occurrence of discrete voiding contractions rather than the relatively slow increase of intravesical pressure that leads to dribbling incontinence as the compliance limits of the bladder are approached.

**Cystometry Methods.** For transvesical cystometry, cats were anesthetized with isoflurane. Throughout the remainder of the surgical manipulations, anesthesia was gradually supplemented with 60 mg/kg i.v. $\alpha$-chloralose (Sigma-Aldrich, St. Louis, MO) as isoflurane was gradually reduced. The $\alpha$-chloralose solution was made up as 0.5 g of $\alpha$-chloralose wetted with 0.8 ml of absolute ethanol, then brought up to 10 ml in polyethylene glycol (mol. wt. 400) and dissolved with heating and sonicating. Additional $\alpha$-chloralose was added as needed to maintain stable anesthesia throughout the experiment.

A midline incision was made in the lower abdomen to expose the bladder and a cannula was inserted into the bladder through the dome. The cannula was connected to PE-90 tubing which was connected to a model 975 syringe pump (Harvard Apparatus Inc., Holliston, MA) for continuous infusion of saline and to another pressure transducer for measurement of intravesical pressure. The rate of saline infusion varied from animal to animal to maintain a practical and consistent time in reaching bladder capacity. The bladder capacity (i.e., volume of saline infused into the initially empty bladder before triggering a voiding contraction) varied from 1.8 to 7.1 ml in intact cats and from 6.4 to 112.3 ml in SCI cats. We chose to adjust the saline infusion rate before drug treatment was begun so that bladder capacity was reached in about 5 to 10 min. The carotid artery was cannulated with PE-90 tubing which was connected to a pressure transducer (Ohmeda Model P23XL-1; Gould Instrument System Inc., Cleveland, OH) for measurement of arterial pressure and heart rate.

To record the electromyogram of the external urethral sphincter (EUS-EMG), 0.005-inch-diameter Medwire AG57 polyesterfluorocarbon-coated silver wire electrodes (Sigmund Cohn Corp., Mount Vernon, NY) were inserted percutaneously into the EUS. This procedure was performed using a 25 gauge needle with a hooked EMG electrode positioned at the tip. The needle was inserted adjacent to the sphincter, approximately 5 mm lateral to the urethral meatus, and then withdrawn, leaving the EMG wires embedded in the muscle. The electrodes were connected through a Model H15P high impedance probe (Grass Instruments, Quincy, MA) to a Grass P5 amplifier.

A modified plastic pipette connected to a vacuum catheter at its narrow end was positioned at the urethral meatus to collect voided solution during micturition. The vacuum catheter brought voided solution into a syringe hanging from a Model A-934 force transducer (Kulite Semiconductor Products Inc., Leonia, NJ), which was used to measure micturition volume, assuming the specific gravity to be approximately 1.

After obtaining at least three reproducible free-running (continuous infusion) micturition cycles (i.e., continuous infusion with rhythmic bladder contractions accompanied by urine release), vehicle or drug was administered and the ensuing response observed for a few more free-running cycles to allow time for drug distribution and
assess the onset of drug-induced changes. At that point, the bladder was emptied and three reproducible filling cystometrograms were obtained at each vehicle or drug dosage before returning to free-running conditions. Each cystometrogram ended with the onset of voiding. In spinally intact and chronic SCI cats, voiding contractions are preceded by a variable number of premicturition contractions of two types: low-amplitude myogenic prodromal contractions (Satchell et al., 1993) and larger nonvoiding contractions which occur after mechanoreceptor activation and are thought to be neurogenic (Satchell, 1991). We used an arbitrary amplitude of 10 mm Hg to distinguish myogenic prodromal from neurogenic nonvoiding contractions. The pressure and volume data were sampled at 50 Hz and the EUS-EMG data were sampled at 5000 Hz using a PCI-MIO-16-XE10 data acquisition board (National Instruments, Austin, TX), stored in a computer, and analyzed using a program written for that purpose in the LabVIEW environment (National Instruments, Austin, TX). Measurements were made of micturition volume (amount released by the bladder through the urethra), residual volume (volume remaining in the bladder after completion of a micturition), capacity (micturition volume plus residual volume; i.e., the volume infused to trigger the micturition reflex), threshold volume (volume infused up to the occurrence of the first contraction exceeding 10 mm Hg in amplitude, including both nonvoiding and voiding contractions), peak intravesicular pressure, heart rate, and systolic, diastolic, and mean arterial pressures.

**Drugs.** 8-OH-DPAT (Sigma-Aldrich) was used as a 5-HT1A agonist. In our previous study (Thor et al., 2002), we used a racemic (R,S) mixture. Some data indicate a difference in the effectiveness of (R)- and (S)-enantiomers (Bjork et al., 1989; Cornfield et al., 1991); however, our preliminary studies (not shown) revealed no cystometic difference. 8-OH-DPAT and WAY-100635 (Sigma-Aldrich) were dissolved in distilled water. GR-46611 (Tocris Cookson Inc., Ellisville, MO) was dissolved in 20 mM acetic acid. All animals were euthanized after cystometry with Fatal Plus (0.5 ml i.v.).

In each dose–response curve figure, the leftmost value is the average obtained with vehicle treatment for that group. The value at each drug dose equals the vehicle average plus the estimated average effect of the dose relative to vehicle. These estimated dose effects, along with their displayed standard errors, are obtained from the linear regression. The standard errors are appropriate for testing hypotheses that average values at drug doses differ from average values with vehicle alone. Vehicle averages are not plotted with standard errors because comparisons of vehicle against vehicle are meaningless. However, vehicle averages are given with conventional estimates of the standard error of the mean in Table 1.

Data analysis was performed using S-Plus v. 6.0 for Windows (Insightful, Seattle, WA). In figures showing the results of statistical tests, * = significant at *p* < 0.05, ** = significant at *p* < 0.01, and *** = significant at *p* < 0.001 (with Bonferroni correction as appropriate).

**Tables and Graphical Presentations.** To estimate the differential effects of doses and their standard errors, we fit linear regressions with indicator variables for “cat effects” (i.e., interanimal differences in bladder characteristics) and dose effects (Neter et al., 1996). This is equivalent to a repeated measures analysis of variance (ANOVA). The fitted regression model are identical to those from an analysis of variance (ANOVA) that controls for cat effects. Such control is essential, because the variation in outcomes across cats swamps the variation of outcomes that controls for cat effects. For example, the predrug values of bladder capacity ranged from 5 to 116 ml in different SCI cats. Regression models were fit separately for each of the three groups of cats (intact with 8-OH-DPAT, SCI with 8-OH-DPAT, and SCI with GR-46611) using all observed data. The sums of squares and hence the *F* statistic from the fitted regression model are identical to those from an analysis of variance that blocks by animal. The overall *F* tests in our regression models, which tested the analysis of variance null hypothesis that all doses have zero effects, had very small *F* tests with Bonferroni corrections for the number of dose levels.

**Results**

**Effects of 8-OH-DPAT on Intact Cats.** Among intact cats (*n* = 5), the filling cystometrogram in the absence of 8-OH-DPAT showed a variable number of nonvoiding contractions followed by a single- or multi-peaked voiding contraction. The cystometric response to 8-OH-DPAT, summarized in Fig. 1, was variable. Some cats showed increases in threshold volume, bladder capacity, and residual volume that were similar to those seen in SCI cats (see below), whereas others showed minor or inconsistent responses. These variable results are very similar to previous studies (Thor et al., 2002).

**Effects of 8-OH-DPAT on SCI Cats.** Cystometrograms from a dose–response study of a representative SCI cat are shown in Fig. 2, in which each cystometrogram stops at the time of micturition. With increasing doses of 8-OH-DPAT, the time to the first contraction is increased as well as the time to micturition. As summarized in Fig. 3 for all SCI cats studied with 8-OH-DPAT (*n* = 6), treatment with increasing doses resulted in a progressive increase in the threshold volume, bladder capacity, and residual volume. Micturition volume and peak intravesicular pressure were not significantly affected at any dose. The 5-HT1A antagonist WAY-100635 was administered to three cats after completion of the 8-OH-DPAT dose–response study. As shown in Fig. 4, WAY-100635 caused a partial reversal of the increase in threshold volume and a nearly complete reversal of the increases in capacity and residual volume attending treatment with 30 µg/kg 8-OH-DPAT; all of these reversals were significant (*p* < 0.05).

The cystometrogram of one SCI cat was characterized by nearly continuous nonvoiding contractions beginning shortly after infusion was started (Fig. 5); urine was not released until the bladder appeared to reach the limit of its capacity.

**Table 1**

Baseline cystometric values for the three study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Threshold Volume</th>
<th>Micturition Volume</th>
<th>Residual Volume</th>
<th>Bladder Capacity</th>
<th>Bladder Pressure</th>
<th>Bladder Mass</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ml</td>
<td>ml</td>
<td>ml</td>
<td>mm Hg</td>
<td>g</td>
<td>g</td>
</tr>
<tr>
<td>Intact-DPAT</td>
<td>4.1 ± 1.1</td>
<td>1.6 ± 0.4</td>
<td>3.3 ± 1.1</td>
<td>4.9 ± 1.0</td>
<td>22.1 ± 1.8</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>SCI-DPAT</td>
<td>8.6 ± 1.8</td>
<td>1.4 ± 0.5</td>
<td>41.2 ± 21.6</td>
<td>42.5 ± 21.6</td>
<td>24.8 ± 2.3</td>
<td>6.7 ± 1.1</td>
</tr>
<tr>
<td>SCI-GR</td>
<td>6.0 ± 2.0</td>
<td>1.2 ± 0.2</td>
<td>7.0 ± 3.1</td>
<td>8.3 ± 3.1</td>
<td>33.8 ± 6.1</td>
<td>5.3 ± 0.5</td>
</tr>
</tbody>
</table>

The three study groups are intact and SCI cats studied with 8-OH-DPAT (Intact-DPAT and SCI-DPAT, respectively), and SCI cats studied with GR-46611 (SCI-GR). Bladder mass values for intact cats were obtained from 15 cats of the same age and body mass, which were used in other studies after this study was completed. All values are mean ± S.E.M.
Intact cats given 8-OH-DPAT

A  threshold volume

B  capacity

C  residual volume

8-OH-DPAT (μg/kg, i.v.)  8-OH-DPAT (μg/kg, i.v.)  8-OH-DPAT (μg/kg, i.v.)

Fig. 1. Dose-response curves for 8-OH-DPAT in intact cats. The leftmost value (veh) is the average vehicle response. Estimated dose effects from linear regression and their standard errors are displayed; vehicle response is not plotted with standard error bars because comparisons of vehicle against vehicle are meaningless. Bars indicate standard error. *, significant at \( p < 0.05 \); **, significant at \( p < 0.01 \) (with Bonferroni correction as appropriate).

Fig. 2. Dose-response curve cystometrograms for 8-OH-DPAT in an individual SCI cat. Micturition occurred at the point when each cystometrogram ends. Increase in threshold volume and capacity with 8-OH-DPAT and reversal with WAY-106635 are apparent.

This cat had the largest cystometric capacity and the second largest bladder mass of all the cats studied. This was the sole chronic SCI cat that did not show discrete voiding contractions and in which a post-transection spinal micturition reflex may not have emerged. No significant change in any cystometric variable was found with 8-OH-DPAT treatment except that the nonvoiding contractions were eventually eliminated (Fig. 5), which did not occur in any other cat. Because none of the nonvoiding contractions exceeded 10 mm Hg in magnitude, this cat did not yield threshold volume data for inclusion in Fig. 3.

Effects of GR-46611 on SCI Cats. Among SCI cats treated with GR-46611 \((n = 7)\), increasing doses of GR-46611 resulted in a statistically significant dose-related increase in threshold volume, bladder capacity, and residual volume (Figs. 6 and 7). Micturition volume and peak intravesicular pressure were not significantly affected. Because GR-46611 has considerable affinity for the 5-HT\(_{1A}\) receptor (Barf et al., 1996), the selective 5-HT\(_{1A}\) receptor antagonist WAY-100635 was administered after the last dose of GR-46611 to three of these seven cats. As shown in Figs. 6 and 8, WAY-100635 had no significant effect on the increases in threshold volume, capacity, and residual volume induced by the maximal dose of GR-46611.

EUS-EMG Activity. EMG recordings in these cats showed very little activity. In many, but not all, cats, brief low-amplitude activity was seen at the time of micturition contractions (and generally not at the time of nonvoiding contractions). Other types of activity included occasional activity associated with visible contractions of the perineal, abdominal, or hindlimb musculature, which showed no obvious correlation to cystometric events, as well as occasional activity induced by withdrawal of infused from the bladder as part of filling cystometry. Neither 8-OH-DPAT nor GR-46611 produced increases in EUS-EMG activity.

Discussion

We hypothesized that in chronic SCI cats (and humans), in which C fibers initiate the micturition reflex, the 5-HT\(_{1A}\) receptor agonist 8-OH-DPAT might increase both bladder capacity and EUS-EMG activity just as it does when C fibers are activated by acetic acid infusion of the bladder in cats with an intact spinal cord (Thor et al., 2002). We further hypothesized that the 5-HT\(_{1B/1D}\) agonist GR-46611 would increase bladder capacity. Our hypotheses were borne out for bladder capacity, but not for EUS-EMG activity.

8-OH-DPAT and Bladder Capacity in Intact Cats. Cats with intact spinal cords whose bladders were infused with saline showed a trend toward an increase in bladder capacity with increasing doses of 8-OH-DPAT, as previously reported (Thor et al., 2002). However, that trend was not statistically significant in our prior study and was irregularly significant in the current study. This seems to be the consequence of both intra- and interanimal variability in response to the 5-HT\(_{1A}\) agonist. Some of this variability might arise.
from the dual role of 5-HT\textsubscript{1A} receptors as both presynaptic autoreceptors and postsynaptic receptors. Thus, intravenous administration of 8-OH-DPAT will have effects not only on 5-HT\textsubscript{1A} receptors of primary afferent neurons and spinal cord neurons involved in micturition, but also on inhibitory 5-HT\textsubscript{1A} autoreceptors of serotonin-producing neurons of the midbrain. The consequence of action upon the autoreceptors is a decrease in spinal cord serotonin release that would thereby reduce serotonin binding at multiple 5-HT receptor subtypes, including the 5-HT\textsubscript{1A} subtype.

**Effects of 8-OH-DPAT and GR-46611 on Micturition in SCI Cats.** As shown in Fig. 3, 8-OH-DPAT significantly increased threshold volume, bladder capacity, and residual volume in SCI cats. Although 8-OH-DPAT binds to both 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} receptors (Jasper et al., 1997; Thomas et al., 1998), the nearly complete reversal of 8-OH-DPAT effects by the 5-HT\textsubscript{1A}-selective antagonist WAY-100635, shown in Figs. 2 and 4, clearly indicates that the 5-HT\textsubscript{1A} receptors are critically important for the micturition-related effects of 8-OH-DPAT. GR-46611 also significantly increased threshold volume, bladder capacity, and residual volume in SCI cats (Figs. 6 and 7). The effectiveness of the 5-HT\textsubscript{1} receptor agonists in increasing bladder capacity and decreasing threshold volume is in line with the well known antinociceptive properties of serotonin (Millan, 1997), and their abundance in the dorsal horn of the spinal cord (Thor et al., 1993; Millan, 1997) is in accord with their hypothesized action on the sensory limb of the micturition reflex.

GR-46611 is a less selective agonist than 8-OH-DPAT, binding not only to 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors with nearly equal affinity, but also to 5-HT\textsubscript{1A} receptors with an affinity approximately 5 times lower (Barf et al., 1996). Accordingly, it seemed plausible that its micturition-related effects could have been mediated through the 5-HT\textsubscript{1A} receptor; however,
SCI cats given GR-46611

A threshold volume

B capacity

C residual volume

Fig. 7. Dose-response curves for GR-46611 in SCI cats. Bars indicate standard error. *, significant at $p < 0.05$; **, significant at $p < 0.01$; and ***, significant at $p < 0.001$ (with Bonferroni correction as appropriate).

Fig. 8. Lack of reversal of GR-46611 effects by WAY-100635 in three cats. The gray bars show the response to the highest dose of GR-46611 as percentage of vehicle; the black bars show the effect of WAY-100635 administered after the highest dose of GR-46611. Compare with Fig. 4. In all cases, WAY-100635 is without significant effect.

Fig. 9. Effect of 5-HT1 agonists on mean arterial pressure (MAP) in intact and SCI cats. GR-46611 was administered only to SCI cats. Bars indicate standard error. *, significant at $p < 0.05$; **, significant at $p < 0.01$; and ***, significant at $p < 0.001$ (with Bonferroni correction as appropriate).

the nearly complete ineffectiveness of WAY-100635 at reversing GR-46611 actions (Fig. 8) argues against that hypothesis.

5-HT1 Agonists and the EUS-EMG. In previous studies in cats with intact spinal cords, 8-OH-DPAT dramatically increased EUS-EMG activity when the bladder was infused with 0.5% acetic acid to activate bladder nociceptors and irritate the bladder (Thor et al., 2002). Because both nociceptive infusion in intact cats and innocuous infusion in SCI cats use C fibers for signaling, we expected that 8-OH-DPAT would increase EUS-EMG activity in SCI cats with saline infusion. However, no such effect was shown.

That we failed to observe heightened EUS-EMG activity despite a pronounced effect on bladder capacity (Fig. 3) cannot be blamed on the use of $\alpha$-chloralose as an anesthetic because the same anesthetic was used in the previous study of irritated bladder in intact cats (Thor et al., 2002). Changes in the properties of C fibers or their targets due to post-SCI plasticity (Yoshimura et al., 1998, 1999) could be invoked, although our unpublished studies using nociceptive infusion in SCI cats with the same anesthetic usually revealed prominent EUS-EMG activity.

The intensity of nociceptor signaling may be the key variable. Thus, although both 0.5% acetic acid in intact cats and saline infusion in SCI cats presumably use C fibers for signaling, either the rate of C fiber activity or the number of C fibers recruited may be higher with nociceptive infusion of intact cats than with saline infusion of SCI cats. The integrated C fiber signal may then be sufficient to promote EUS-EMG activity in the former case but insufficient in the latter case. Alternatively, there may be two populations of C fibers in SCI cats, one that is mechanosensitive and whose inputs to the EUS are not sensitive to 5-HT1A receptor agonists, and one that is chemosensitive and whose inputs to the EUS are sensitive to 5-HT1A receptor agonists. C fibers can be divided by many characteristics into at least two populations [e.g., peptidergic versus nonpeptidergic (Snider and McMahon, 1998) and nociceptive versus mechanoreceptive (Lawson, 2002)]. Studies with dilute acetic acid infusion into the spinally intact and chronic SCI cat bladder are underway in our laboratory to clarify these issues.

Mean Arterial Pressure. The lower mean arterial pressure in SCI than in intact cats is expected based on the lower vascular tone in caudal regions in SCI cats. The decline in mean arterial pressure with administration of 8-OH-DPAT to both intact and SCI animals (Fig. 9) is expected because 5-HT1A agonists acting centrally cause both decreased sym-
pathetic outflow and increased parasympathetic outflow (Ramage, 2001). The depressant effect of 8-OH-DPAT on cardiovascular function is known to be reversed by WAY-100635 (Wang and Ramage, 2001), as it was here (Fig. 4). It has also been found that central 5-HT1B receptors induce a rise in blood pressure even as central 5-HT1D receptors may cause a decline (Ramage, 2001), which together might explain the rising and then falling shape of the GR-46611 dose-response curve for mean arterial pressure (Fig. 9). The decline is unlikely to be due to the affinity of GR-46611 for 5-HT1A receptors because it was not reversed by WAY-100635 (Fig. 8).

The hypotensive effect of intravenously administered 5-HT1 receptor agonists imposes limits on their utility in cats. Nonetheless, a preliminary report noted achievement of clinically relevant urologic endpoints in chronic SCI cats with subcutaneous administration of 8-OH-DPAT (Miscik et al., 2003). As regards humans, 5-HT1A agonists have been found to induce slight (Lechin et al., 1998) or no significant hypotension (Kahn et al., 1994; Ohman et al., 2001), whereas 5-HT1B/D agonists have been found to induce hypertension (Vannmolkot et al., 2002) or no significant change in blood pressure (Visser et al., 1996). The full prescribing information for orally administered buspirone (5-HT1A agonist) lists hypotension as a rare adverse reaction, whereas that for orally administered sumatriptan (5-HT1B/1D agonist) lists both hypertension and hypotension as adverse reactions. Overall, it appears that humans tolerate 5-HT1 agonist treatment better than cats do, or perhaps that chronic oral dosing produces less hypotensive effect than bolus intravenous dosing.

In summary, 5-HT1 receptors may offer therapeutic targets for controlling bladder hyperreflexia in patients with spinal cord injury. This offers the potential of improving continence, reducing renal perfusion pressure, and reducing autonomic dysreflexia.

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References

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