Reduction of spinal sensory transmission by facilitation of 5-HT<sub>1B/D</sub> receptors in noninjured and spinal cord-injured humans

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¹Department of Biomedical Engineering, University of Alberta, Edmonton, Alberta, Canada; ²Centre for Neuroscience, University of Alberta, Edmonton, Alberta, Canada; ³Faculty of Rehabilitation Medicine, University of Alberta, Edmonton, Alberta, Canada; and ⁴Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Alberta, Canada

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D’Amico JM, Li Y, Bennett DJ, Gorassini MA. Reduction of spinal sensory transmission by facilitation of 5-HT<sub>1B/D</sub> receptors in noninjured and spinal cord-injured humans. J Neurophysiol 109: 1485–1493, 2013. First published December 5, 2012; doi:10.1152/jn.00822.2012.—Activation of receptors by serotonin (5-HT<sub>1</sub>) and norepinephrine (α<sub>2</sub>) on primary afferent terminals and excitatory interneurons reduces transmission in spinal sensory pathways. Loss or reduction of descending sources of serotonin and norepinephrine after spinal cord injury (SCI) and the subsequent reduction of 5-HT<sub>1A</sub> receptor activity contributes, in part, to the emergence of excessive motoneuron activation from sensory afferent pathways and the uncontrolled triggering of persistent inward currents that depolarize motoneurons during muscle spasms. We tested in a double-blind, placebo-controlled study whether facilitating 5-HT<sub>1B/D</sub> receptors with the agonist zolmitriptan reduces the sensory activation of motoneurons during an H-reflex in both noninjured control and spinal cord-injured participants. In both groups zolmitriptan, but not placebo, reduced the size of the maximum soleus H-reflex with a peak decrease to 59% (noninjured) and 62% (SCI) of predrug values. In SCI participants we also examined the effects of zolmitriptan on the cutaneomuscular reflex evoked in tibialis anterior from stimulation to the medial arch of the foot. Zolmitriptan, but not placebo, reduced the long-latency, polysynaptic component of the cutaneomuscular reflex (first 200 ms of reflex) by ~50%. This ultimately reduced the triggering of the long-lasting component of the reflex (500 ms poststimulation to end of reflex) known to be mediated by persistent current in the motoneuron. These results demonstrate that facilitation of 5-HT<sub>1B/D</sub> receptors reduces sensory transmission in both monosynaptic and polysynaptic reflex pathways to ultimately reduce long-lasting reflexes (spasms) after SCI.

serotonin; H-reflex; cutaneomuscular; spasticity; zolmitriptan

After a complete spinal cord injury (SCI), levels of serotonin and norepinephrine below the lesion decrease greatly because the major supply of these neuromodulators comes from descending sources of serotonin and norepinephrine after spinal cord injury (SCI) and the subsequent reduction of 5-HT<sub>1A</sub> receptor activity contributes, in part, to the emergence of excessive motoneuron activation from sensory afferent pathways and the uncontrolled triggering of persistent inward currents that depolarize motoneurons during muscle spasms. We tested in a double-blind, placebo-controlled study whether facilitating 5-HT<sub>1B/D</sub> receptors with the agonist zolmitriptan reduces the sensory activation of motoneurons during an H-reflex in both noninjured control and spinal cord-injured participants. In both groups zolmitriptan, but not placebo, reduced the size of the maximum soleus H-reflex with a peak decrease to 59% (noninjured) and 62% (SCI) of predrug values. In SCI participants we also examined the effects of zolmitriptan on the cutaneomuscular reflex evoked in tibialis anterior from stimulation to the medial arch of the foot. Zolmitriptan, but not placebo, reduced the long-latency, polysynaptic component of the cutaneomuscular reflex (first 200 ms of reflex) by ~50%. This ultimately reduced the triggering of the long-lasting component of the reflex (500 ms poststimulation to end of reflex) known to be mediated by persistent current in the motoneuron. These results demonstrate that facilitation of 5-HT<sub>1B/D</sub> receptors reduces sensory transmission in both monosynaptic and polysynaptic reflex pathways to ultimately reduce long-lasting reflexes (spasms) after SCI.

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pinprick sensation of lower leg: check mark indicates preserved sensation from tested area of knee downward.

...EPSP produced during a monosynaptic reflex is very short, ~5–10 ms in rat and cat motoneurons (Baker and Chandler 1987; Edwards et al. 1989; Jimenez et al. 1991; Li et al. 2004b), and is estimated to be ~30 ms in humans on the basis of motor unit recordings (Miles et al. 1989). Thus depolarization of the motoneuron during an H-reflex is too brief (<50 ms) to activate a CaPIC, and any reduction in motoneuron output from zolmitriptan would likely be due to a reduction in its sensory synaptic activation. We also examined in SCI participants whether long-lasting polysynaptic reflexes activated by cutaneous muscle stimulation to the medial arch of the foot, as well as the subsequent long-lasting reflexes (spasms) they trigger, were also reduced by zolmitriptan to determine if facilitation of 5-HT1B/D receptors is a potential strategy to reduce spasticity after SCI. Some of these data have been published in abstract form (D’Amico and Gorassini 2012).

**METHODS**

Experiments were approved by the Health Research Ethics Board at the University of Alberta and conformed to the Declaration of Helsinki. The off-label use of the antimigraine drug zolmitriptan in noninjured and SCI participants was approved by Health Canada Clinical Trials. All participants gave written, informed consent before participating in the study. In total, six noninjured control (35 ± 13 yr, 2 female) and seven SCI participants with motor complete injuries (35 ± 10 yr, 2 female) took part in the study (Table 1). Three of the seven SCI participants (subjects 1M–3M in Table 1) also took part in the 5-HT2 receptor study of D’Amico et al. (2012). Drug screens were performed to rule out contraindications for zolmitriptan and to ensure participant safety. Five other SCI participants were excluded from the study due to drug contraindications from antidepressants, and one subject was excluded due to a blood clotting disorder that made it unsafe to administer zolmitriptan. An additional two SCI participants were excluded from the study (both motor complete, T11–T12 and T6–T7) because appreciable H-reflexes could not be evoked from either leg.

**Drug administration.** All participants were required to come to the laboratory on two separate occasions (separated by at least 1 wk) to receive placebo or the drug zolmitriptan in random order. Drug and placebo were housed in a two-part telescoping capsule to conceal the identity of the drug. Noninjured control and SCI participants received a 10-mg dose of the 5-HT1B/D agonist zolmitriptan (Proietti-Cecchini et al. 1997; Visser et al. 1996; Werhahn et al. 1998). Placebo was a sugar pill with similar weight to the zolmitriptan tablets. JDA, who performed the data analysis, was also blinded until data analysis was completed. Heart rate and blood pressure were measured before and every 60 min after drug intake. Participants were asked to report any physiological sensations experienced after taking zolmitriptan or placebo. Because plasma concentrations of zolmitriptan are detectable at 15 min after oral intake with peak concentrations occurring at 2–4 h (Peterlin and Rapoport 2007), reflex recordings were taken every 30 min for 2 h after drug intake. This allowed us to examine the onset of the drug effect and make measurements near the time of peak plasma concentrations. In pilot experiments, taking reflex recordings beyond 2 h after drug intake was too fatiguing for the participants.

**H-reflex recordings.** H-reflex recordings were obtained in both noninjured and SCI participants. H-reflexes were evoked in the soleus muscle because they are readily elicited at rest, which was important for the SCI participants since they could not produce voluntary contractions. All noninjured participants, and SCI participants who were able to transfer safely, were placed in a supine position on a padded table. Two SCI participants were examined in a reclined position in their powered wheelchair (subjects 1M and 4F in Table 1). Two surface electrodes (2.2 × 3.3 cm; Kendall Soft-E, Chicopee, MA) were placed over the right soleus muscle to record electromyography (EMG) signals. The soleus H-reflex was evoked by stimulating the tibial nerve (DS7A constant-current stimulator NL703; Digitimer, Welwyn Garden City, UK) through a monopolar electrode once the best position was found with a probe electrode (1-ms pulse width, return electrode placed over patella). The surface EMG signal was amplified 200 or 1,000 times (depending on the size of the response) and filtered using a bandpass of 20–2,500 Hz (model 2024F; Intronix Technologies, Bolton, ON, Canada). All signals were digitized at a rate of 5 kHz using Axonoscope hardware and software (Digidata 1440 Series; Molecular Devices, Sunnyvale, CA) and stored on a personal computer for off-line analysis.

**H-reflex recruitment curves.** H-reflex responses were evoked at incrementing stimulus intensities to produce a recruitment curve before (2 baseline curves) and 30, 60, 90, and 120 min after drug/placebo intake. Before each H-reflex recruitment curve was produced, the motor threshold (MT) was determined online as the stimulation intensity required to elicit an M-wave of ~100 μV. The stimulation intensity was expressed as a multiple of motor threshold (×MT) and was set from below H-reflex threshold (ranging from 0.5 to 0.7 ×MT) to when the H-reflex decreased after its peak (ranging from 1.2 to 1.6

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| Description of spinal cord-injured (SCI) participants including age of participant and age of injury at the time of experiment, injury level, ASIA Impairment Scale (AIS), cause of injury, Modified Ashworth Score (MAS; 0–4), and Penn Spasm Frequency Scale (0–4). Final columns describe spared light touch and pinprick sensation of lower leg: check mark indicates preserved sensation from tested area of knee downward.
×MT) in steps of 0.05 × MT. This ensured that a minimum of eight to nine points were collected along the steep portion of the H-reflex recruitment curve. Five reflexes were evoked at each stimulation intensity. Stimuli were delivered every 3 s, which allowed enough time to manually increase the stimulation intensity after every fifth trial. The maximal motor response (M_max) was measured after each recruitment curve.

H- and M-wave amplitudes were measured as peak to peak using custom-written software in Matlab (The MathWorks, Natick, MA). The five H- and M-wave amplitudes, evoked at each stimulation intensity, were averaged together and normalized to M_max. The amplitude of the normalized H- and M-waves was plotted at each stimulation intensity, with the latter expressed as a function of MT, to produce H- and M-wave recruitment curves. To standardize the measurement of MT across the different time points and participants, MT was recalculated off-line using the x-intercept method (as per Kerr and Vujnovich 2002; Lundbye-Jensen and Nielsen 2008). Briefly, the steep portion of the M-wave recruitment curve was fitted with a straight line, and its x-intercept was calculated as the new MT, producing better alignment of the M-wave recruitment curves. The H-reflex recruitment curve (up to its peak) was fitted with a three-parameter sigmoid function: $H(s) = H_{\text{max}} / [1 + e^{-(s-S_50)/a}]$ (Klimstra and Zehr 2008). The peak H-reflex (H_peak) and the stimulation intensity producing 50% of H_max (S_50) were measured off the fitted curve. The slope parameter a was too variable because it depended on the number of points along the recruitment curve, and therefore it was not analyzed (see also Klimstra and Zehr 2008). Typically, 98% of the variance in the H-reflex recruitment curve was accounted for by the sigmoidal fit with $r^2$ values ranging from 0.92 to 0.99 (median = 0.99) in noninjured controls and from 0.87 to 0.99 (median = 0.98) in SCI participants. The threshold to evoke an H-reflex (H_threshold) was measured as the stimulus intensity required to elicit an H-reflex that was 5% of H_max. Because there was large variability in the size of H-reflexes between SCI participants, the three parameters of the H-reflex recruitment curve (H_max, S_50, and H_threshold) at the 30-, 60-, 90-, and 120-min time points were expressed as a percentage of the predrug value and averaged across subjects. Cutaneousmuscular reflex recordings. Cutaneousmuscular reflexes were recorded in SCI participants only because long-lasting responses (>1 s) cannot be evoked in noninjured control participants. Cutaneousmuscular reflexes were evoked in the tibialis anterior (TA) muscle because it has previously been shown that long-lasting responses, likely mediated by CaPIC activation, are readily produced in the TA after SCI (Norton et al. 2008). Cutaneousmuscular afferents supplying the side and sole of the foot were stimulated with long pulse trains applied to the medial arch of the foot (300 Hz, 14 pulses, 0.5-ms pulse width; DSTA constant-current stimulator) at an intensity that was just below pain threshold (40 ± 15 mA on average). Surface EMG signals from the TA were amplified 1,000 times and filtered using a bandpass of 20–2,500 Hz (model 2024F; IntroniX Technologies). Both limbs were tested, and the TA muscle that exhibited the longest reflex response predrug was used. In most SCI participants, the right TA had the longest responses, except for participants 2M and 5M. Stimulation was repeated 6 times every 6 s for each trial. Three to four predrug reflex responses were recorded until two consecutive responses fell within 10% of each other. These last two predrug reflex responses were averaged together to form the baseline reflex response. Cutaneousmuscular reflex recordings were repeated at 30, 60, 90, and 120 min after drug intake and were performed immediately after each H/M recruitment curve.

Cutaneousmuscular reflex analysis. The cutaneousmuscular reflex was divided into two components: a long-latency polysynaptic reflex (LPR) and a long-lasting reflex (LLR) as per Murray et al. (2010, 2011) and Rank et al. (2011). The LPR, which includes the start of the reflex response up until 300 ms after the first stimulation pulse, contains a mixture of both sensory-evoked EPSPs and CaPIC activation, because its amplitude is reduced to ~50% by the Ca\textsuperscript{2+} channel blocker isradipine (Li et al. 2004a; Murray et al. 2011). The average latency of the LPR was 84 ± 14 ms, with an average duration of 215 ± 15 ms. The later, long-lasting reflex component (LLR) was defined as the time window from 500 ms after the first stimulation pulse to the end of the reflex response in the predrug trial, as per Murray et al. (2010, 2011) and Rank et al. (2011) (LLR duration: 400 ± 158 ms). Thus the LLR represents a period where most of the sensory synaptic drive to the motoneuron (i.e., EPSP) has subsided and is produced mainly by a depolarization from the CaPIC (Li et al. 2004a; Norton et al. 2008).

In Matlab, each EMG trace was first rectified and the mean EMG was calculated for the time windows of the two reflex components (LPR: start of reflex to 300 ms poststimulation; LLR: 500 ms post-stimulation to end of predrug response). The mean rectified background noise, measured from 100 ms before the stimulation, was subtracted from the data. The mean EMG values for each of the six sweeps were averaged together to obtain LPR and LLR values for each time point. All values were expressed as a percentage of the predrug value and then averaged across subjects for each experimental session (zolmitriptan or placebo).

In vitro monosynaptic and polysynaptic reflex recordings. To examine the effects of zolmitriptan applied directly to the spinal cord on monosynaptic reflexes that are similar to the H-reflexes recorded in our human participants, we used the in vitro sacral spinal cord preparation (Bennett et al. 2001a; Li and Bennett 2003). Under urethane anesthesia (1.8 g/kg), the whole spinal cord caudal to S2 (sacral) was removed from chronic spinal rats and immersed in oxygenated artificial cerebrospinal fluid (ACSF; flowing 8 ml/min); recordings were made starting 2.5 h later, as detailed previously (Bennett et al. 2001a; Li and Bennett 2003). Ventral (S4 and Co1, coccycgeal) and dorsal (Co1) roots were mounted on silver wires above the ACSF and covered with Vaseline. The dorsal root was stimulated with a single pulse (0.1 ms, 0.02 mA; ~3 times sensory afferent threshold; repeated 5 times every 10 s for 1 trial, with trials repeated every 12 min). With this stimulation, a monosynaptic reflex of a latency of 2 ms and lasting for ~4 ms was evoked in the ventral roots. A 300 nM dose of zolmitriptan (AstraZeneca, Mississauga, ON, Canada) was used, a dose in the whole sacral spinal cord that is known to reduce polysynaptic EPSPs (Murray et al. 2011).

Statistical analysis. All statistical analysis was performed using SigmaPlot 11.0 software. Values are expressed in the text as means ± SD and in Figs. 2 and 3 as means ± SE. Normality for the parameters of the H-reflex recruitment curve (H_max, S_50, and H_threshold) and for the LPR and LLR components of the cutaneousmuscular reflexes was first tested with the Shapiro-Wilk test. For each separate experiment (placebo or zolmitriptan), a one-way repeated-measures ANOVA for normally distributed data and a one-way repeated-measures ANOVA on ranks ($\chi^2$ test) for nonnormally distributed data were used to determine if there was an effect of the drug on the reflex parameters over the 30-, 60-, 90-, and 120-min time points. To compare between experiments and to determine whether placebo and zolmitriptan had different effects on the reflex parameters, a two-way repeated-measures ANOVA was used with the within-subject factors drug and time. A post hoc Holm-Sidak test, which corrects for multiple comparisons, was used to determine at which time points the zolmitriptan data differed from the placebo data. Significance was set to $P < 0.05$ in all cases.

RESULTS

Effects of zolmitriptan on the H-reflex recruitment curve. A 10-mg oral dose of the 5-HT\textsubscript{1B/D} receptor agonist zolmitriptan reduced the amplitude of the maximum H-reflex (H_max) in both noninjured and SCI participants. The peak-to-peak amplitude of H_max was reduced 120 min after zolmitriptan intake at similar stimulation intensities to those predrug as reflected in

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the matched M-wave before (black trace) and after (gray trace) drug intake for both noninjured control (Fig. 1A) and SCI participants (Fig. 1C). As shown for the participants in Fig. 1, the average nonnormalized Hmax measured before zolmitriptan intake was significantly larger in controls (2.95 ± 1.36 mV) compared with SCI participants (1.35 ± 1.31 mV, P = 0.05). Likewise, Mmax was larger in controls (6.00 ± 2.50 mV) compared with SCI participants (3.10 ± 1.58 mV, P = 0.03), resulting in Hmax-to-Mmax ratios being similar between the two groups (controls: 0.51 ± 0.17; SCI: 0.42 ± 0.25, P = 0.46).

As shown from the corresponding H-reflex recruitment curves from the these two participants (Fig. 1, B and D), zolmitriptan mainly affected the amplitude of the H-reflex and not its overall excitability, because there were no lateral shifts in the recruitment curve plotted as a function of MT. The reduction in H-reflex size occurred even though the M-wave recruitment curves remained unchanged, signifying a reduction in the transmission of Ia afferent pathways to the soleus motoneuron pool. As in most subjects, the decrease in H-reflex amplitude was most pronounced at 90 and 120 min after drug intake. In four of six noninjured controls and in five of seven SCI participants, the H-reflex was suppressed at all stimulation intensities, as shown for the two participants in Fig. 1, B and D. In the remaining participants, H-reflexes began to decrease near S50, the stimulation intensity producing half of Hmax. H-reflex recruitment curves before zolmitriptan intake (Pre1&2: solid circles, black lines) and at 30 min postdrug (open circles, dark gray line) were reproducible, suggesting that the H-reflex did not spontaneously decrease over time, similar to the recruitment curves at all time points after placebo intake (data not shown).

**Fig. 2.** Group data: Hmax and Mmax after zolmitriptan. A: top, averaged Hmax expressed as a percentage of predrug values, at 30, 60, 90, and 120 min after zolmitriptan (open circles) and placebo (Plb; filled circles) intake in 6 uninjured control participants. Bottom, average Mmax expressed as a percentage of predrug values at all time points after zolmitriptan (open circles) and placebo (filled circles). B: same as in A but for averaged data across the 7 SCI participants. C: peak decrease in Hmax, expressed as a percentage of predrug values, irrespective of time after zolmitriptan intake for both uninjured control (open bar) and SCI participants (filled bar). D: averaged Hmax, expressed as a percentage of predrug values, for 3 uninjured control participants receiving placebo (filled circles, black line), 5 mg of zolmitriptan (filled circles, gray line), and 10 mg of zolmitriptan (open circles, gray line), respectively. Error bars represent means ± SE. *P < 0.05; **P < 0.01; ***P < 0.005.
when the peak decrease in $H_{\text{max}}$ occurring at either of these time points (60, 90 or 120 min) was plotted, the $H_{\text{max}}$ as a percentage of the predrug value was even lower at 58.9 ± 0.1% for controls and 62.3 ± 0.23% for SCI participants (Fig. 2C), with the peak decrease in $H_{\text{max}}$ similar between the two groups ($P = 0.78$). In three preliminary control participants, the dosage of zolmitriptan needed to be at least 10 mg to show a reduction in $H_{\text{max}}$ given that 5 mg, like placebo, did not produce a decrease in $H_{\text{max}}$ (Fig. 2D). Finally, as reflected in the recruitment curves of Fig. 1, there were no changes in $H_{\text{thresh}}$ or $S50$ for noninjured control and SCI participants after zolmitriptan or placebo (all $F > 0.16$, all $P > 0.12$).

**Cutaneomuscular reflex in SCI.** Long-duration reflex responses (spasms) were evoked in the TA muscle in response to a train of pulses (300 Hz, 14 pulses, 0.5-ms pulse width) applied to the medial arch of the foot as shown for the two participants represented in Fig. 3, A and B. Both the long polysynaptic component of the reflex (LPR, marked by gray bar), which is mediated by both sensory-evoked EPSPs and PICs, and the long-lasting reflex component (LLR, marked by black bar), which is mainly mediated by PICs (see METHODS, Cutaneomuscular reflex analysis for rationale) were reduced by zolmitriptan. It was possible to evoke long-duration reflexes in six of the seven motor complete SCI participants. Similar to the H-reflex, zolmitriptan reduced the size of the LPR over time (Fig. 3C), decreasing it to 46.1 ± 0.32% of predrug values at 120 min ($F = 8.92, P < 0.001$). In comparison, there was no decrease of the LPR after placebo intake ($\chi^2 = 7.07, P = 0.13$). A two-way ANOVA revealed a significant drug × time interaction ($F = 5.325, P = 0.004$), with the LPR after zolmitriptan significantly smaller compared with placebo at the 60-, 90-, and 120-min time points ($P < 0.05$). The reduced LPR consequently resulted in a reduced or nearly abolished LLR (spasm) after zolmitriptan ($F = 7.26, P = 0.002$) but not placebo ($\chi^2 = 3.68, P = 0.45$), with the LLR being reduced to 25.0 ± 0.39% of its predrug value at 120 min. Two-way ANOVA revealed a significant drug × time interaction ($F = 3.67, P = 0.026$), with the LLR significantly smaller after zolmitriptan compared with placebo at the 30-, 60-, 90-, and 120-min time points ($P < 0.05$).

**Monosynaptic reflexes after direct application of zolmitriptan to rat spinal cord.** Because zolmitriptan was given orally in the control and SCI participants, this leaves open the possibility that the reduction in H-reflexes could have been due, in part, to systemic actions of the drug on 5-HT$_{1B/D}$ receptors located on blood vessels in the spinal cord, a distinct possibility given that the main clinical use of zolmitriptan is to reduce vasodilatation during migraines (Martin 1997; Peterlin and Rapoport 2007). Therefore, we examined the effects of applying zolmitriptan directly to the spinal cord on monosynaptic reflexes evoked from Co1 dorsal root stimulation in an in vitro sacral spinal cord preparation (see METHODS). When 300 nM zolmitriptan was applied directly to the spinal cord, the amplitude of the monosynaptic reflex was reduced (Fig. 4A), similar to that seen for the H-reflex in human participants (Fig. 1). Zolmitriptan reduced the size of the monosynaptic reflex by 40% or more in five of five rats tested at 15 min after bath application of the drug.

**Short-latency polysynaptic reflexes: rat and human.** In some rats, rather than a monosynaptic reflex, an SPR was evoked in the ventral root that lasted from 10 to 40 ms poststimulation (Fig. 4C) and that was also reduced by zolmitriptan (Fig. 4D; data from Murray et al. 2011). A similar distinct SPR was also evoked in three of the SCI participants during the cutaneomuscular reflex recordings (see also D’Amico et al. 2012). The SPR had a latency of ~70 ms and a duration of 50 ms (Fig. 4E). In all three SCI participants, zolmitriptan reduced the SPR to 56% of its predrug value 120 min after drug intake, as shown for the SCI participant in Fig. 4F (subject 6M, Table 1).

**DISCUSSION**

We have demonstrated that facilitation of 5-HT$_{1B/D}$ receptors with zolmitriptan, but not placebo, reduced sensory transmission to motoneurons as evidenced by the suppression of H-reflexes in noninjured and SCI participants. Likewise, in participants with SCI, zolmitriptan reduced long-latency polysynaptic reflexes evoked by cutaneomuscular stimulation to ultimately reduce the triggering and overall amplitude of long-lasting reflexes (spasms). Although zolmitriptan cannot be taken orally on a daily basis, these results open the possibility that other 5-HT$_{1B/D}$ receptor agonists may be useful to control sensory transmission and reduce the triggering of muscle spasms after SCI.

**Mechanism of action of zolmitriptan on soleus H-reflexes.** In most control and SCI participants (8/13), the H-reflex was reduced at all stimulation intensities, indicating that all reflex pathways, including those with the lowest thresholds, were affected by 5-HT$_{1B/D}$ receptor facilitation. In the remainder of participants (5/13), only H-reflexes activated at stimulation...
Fig. 4. Effects of zolmitriptan verified in a rat model of SCI: A: monosynaptic reflex (Mono) recorded from a ventral root of a chronically spinalized rat that did not display a polysynaptic reflex response. B: monosynaptic reflex from A following 300 nM bath application of zolmitriptan (+Zolm). C: short-latency polysynaptic reflex (SPR) recorded from a ventral root of a different chronically injured rat (no monosynaptic response was evoked). D: monosynaptic reflex from C after 300 nM application of zolmitriptan (modified from Fig. 5 in Murray et al. 2011). E and F: overlays of 6 SPRs recorded from TA in a SCI participant (subject 3M in Table 1) before (E) and 120 min after (F) 10 mg of oral zolmitriptan. In A–F, asterisks mark time of single pulse, or start of multiple pulse, stimulation.

Intensities >S50 were reduced by zolmitriptan, indicating that only the higher threshold sensory pathways were affected in these participants. In all participants, there was a consistent decrease in Hmax at matched amplitudes of M-wave activation, the latter an indirect indication that the number of sensory afferents activated pre- and postdrug was similar (Misiaszek 2003; Zehr 2002). The decrease in Hmax and the absence of any change in Hfres or S50 suggest that facilitation of 5-HT1B/D receptors by zolmitriptan specifically reduced the transmission of sensory-activated inputs in the H-reflex pathway without reducing the excitability of motoneurons (Misiaszek 2003). This finding in the human is in agreement with animal studies where zolmitriptan specifically reduced sensory-evoked EPSPs but did not influence motoneuron properties such as input resistance, resting membrane potential, and spike threshold (Murray et al. 2011). Taken together, this suggests that the reduction in H-reflexes was due to decreases in transmission of sensory pathways to the motoneuron, likely via increases in presynaptic inhibition on terminals of sensory afferents or excitatory interneurons or from postsynaptic inhibition of excitatory interneurons, as a result of 5-HT1B/D receptor facilitation. In addition, because H-reflexes were evoked at a rate of 0.33 Hz, a frequency at which “rate-dependent” or “homosynaptic” depression occurs (Crone and Nielsen 1989), zolmitriptan may have reduced the amplitude of the H-reflexes by facilitating a rate-dependent, inhibitory mechanism.

Mechanism of action of zolmitriptan on cutaneomuscular reflexes. Similar to the Ia-mediated H-reflex pathway, zolmitriptan also reduced short- and long-latency polysynaptic reflexes (SPR and LPR) evoked from cutaneomuscular afferent stimulation in participants with SCI. Reduction of the polysynaptic reflexes was also associated with a reduction in long-lasting reflexes (LLR or spasms). As shown from animal studies with cutaneous reflexes very similar to those of humans, the LPR can last for 500–1,000 ms and is an ~50% mixture of EPSP and PIC activation, whereas the LLC, which lasts for many seconds, is mainly mediated by PIC activation (Murray et al. 2011). It is the long EPSP during the LPR that provides sufficient depolarization of the motoneuron to activate the CaPIC, which then drives self-sustained firing of the motoneuron during a muscle spasm. Because zolmitriptan only reduces sensory activation of the motoneuron and not the PIC (Murray et al. 2011), the reduction in LLC (spasm) activity was likely mediated by the inability of the reduced LPR to trigger a CaPIC and self-sustained firing of the motoneuron. Although we did not estimate the effects of zolmitriptan on PIC activation in this study (e.g., with paired motor unit analysis), we believe a similar mechanism occurred in the human participants with SCI. For instance, results from the H-reflex experiments suggest that sensory transmission, but not motoneuron excitability, was affected by 5-HT1B/D receptor facilitation.

Effect of zolmitriptan on sensory transmission likely via 5-HT1B/D receptors. Zolmitriptan is a commonly prescribed antimigraine medication that is able to cross the blood-brain barrier, although only at relatively high doses (Proietti-Ceccini et al. 1997; Visser et al. 1996; Werhahn et al. 1998). It displays high affinity to 5-HT1B (Ki = 5.01 nM) and 5-HT1D (Ki = 0.63 nM) receptors, with modest affinity to the 5-HT1F receptor (Ki = 63.09 nM) (Martin et al. 1997). Zolmitriptan likely exerts at least some of its effects on the transmission of cutaneomuscular afferent pathways via activation of the 5-HT1B receptor given that in animal studies, the potency of various 5-HT1 receptor agonists in reducing cutaneous polysynaptic reflexes and sensory-evoked EPSPs was correlated to the published binding affinity of only 5-HT1B and 5-HT1D receptor agonists and not to other 5-HT receptor agonists (Murray et al. 2011). Likewise, only 5-HT1B receptor antagonists reversed the effects of zolmitriptan on long-latency polysynaptic reflexes in animals. Thus the activation of 5-HT1B, and not 5-HT1D, receptors, by zolmitriptan likely produced the reduction of cutaneomuscular polysynaptic reflexes evoked in the SCI participants in this study. It remains to be determined in animal studies whether the action of zolmitriptan in reducing the Ia-mediated monosynaptic (H) reflex is also mediated via the 5-HT1B receptor or if there is also involvement of the 5-HT1D receptor to which zolmitriptan has a high binding affinity towards (Honda et al. 2003).

It is interesting that zolmitriptan produced similar decreases in H-reflexes in participants with and without SCI at the 10-mg dose, even though reduced levels of endogenous 5-HT were likely present below the lesion in the SCI participants (Murray et al. 2010). This suggests that after SCI, 5-HT1B/D receptors do not develop supersensitivity to applied agonists, similar to findings in rats where intravenous administration of sumatripan, a similar 5-HT1B/D receptor agonist, depressed the monosynaptic reflex to the same degree in uninjured and SCI
rats (Honda et al. 2006). It would be interesting to examine if other 5-HT receptors, such as 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{7} receptors, which have been shown to help facilitate locomotion after SCI (Antri et al. 2005; Vinay et al. 2012), develop supersensitivity to 5-HT receptor agonists in the presence of reduced levels of endogenous 5-HT.

Clinical implications. Following spinal cord injury, the activation of G_{a} coupled pathways in the motoneuron via constitutive 5-HT_{2}/α_{1} receptors facilitates the activation of CaPICs that mediate, in part, the self-sustained activation of motoneurons during involuntary muscle spasms evoked by brief sensory afferent inputs. This is illustrated in the schematic of Fig. 5, which summarizes the animal and human data from Murray et al. (2010, 2011), Rank et al. (2011) and the previous (D’Amico et al. 2012) and current articles. One strategy to reduce muscle spasms is to reduce 5-HT_{2}/α_{1} receptor activity via inverse agonists such as cyproheptadine (site 2, Fig. 5), resulting in a direct reduction of motoneuron excitability, rather than reducing sensory inputs to the motoneuron like many antispastic drugs used currently (discussed below). Thus the suppression of 5-HT_{2}/α_{1} receptors, specifically 5-HT_{2BC} and α_{1A} receptors, has the potential to reduce excessive muscle activation regardless of the etiology of the spasticity given that the final common pathway, the motoneuron, is directly affected. This strategy would be useful for patients in whom reducing muscle spasticity is a more important goal than preserving residual motor function, such as for patients with motor complete spinal cord injuries or severe brain damage where functional motor movements are lost and spasticity produces painful contractures and joint deformities. These studies highlight the need to develop inverse agonists to 5-HT_{2BC} and α_{1A} receptors that are more specific than cyproheptadine, which has undesirable side effects of drowsiness, histamine receptor activation, and appetite stimulation (Gracies et al. 1997).

In patients with residual motor function, severely reducing motoneuron excitability to alleviate muscle spasticity may not be the best strategy, because this would also reduce activation of the motoneuron by preserved descending inputs. Another strategy to reduce spasticity in this population would be to restore the balance between excitatory and inhibitory activation of the motoneuron by sensory afferent and interneuronal inputs via the activation of G_{i} coupled pathways (site 1, Fig. 5) with GABA_{b} (baclofen: Curtis et al. 1997; Li et al. 2004b), α_{2} (tizanidine: Krach 2011; Meleger 2006), and on the basis of the current study, 5-HT_{1B/D} (zolmitriptan) receptor activation. The main antispastic effect of these drugs is to reduce the sensory-evoked EPSP from direct afferent and interposed interneuronal inputs, allowing an unmasking of an inhibitory postsynaptic potential (IPSP) to ultimately reduce the unchecked activation of CaPICs in the motoneuron (Li et al. 2004b; Murray et al. 2011; Rank et al. 2011). However, all of these G_{i} coupled drugs taken orally have unwanted side effects, such as drowsiness and drug tolerance (Krach 2001; Meleger 2006; Nielsen et al. 2002; Rosche 2002). In addition, zolmitriptan cannot be taken daily due to the risk of harmful by-product production and, ironically, induction of headaches (Martin 1997; Peterlin and Rapoport 2007). Again, this study highlights the need to develop better 5-HT_{1B/D} and possibly 5-HT_{1F}, receptor agonists (Murray et al. 2011) with fewer side effects than baclofen or tizanidine. Alternatively, a moderate suppression of motoneuron activity by cyproheptadine (Wainberg et al. 1990), combined with suppression of sensory inputs, may also strike a proper balance between spasticity control and preservation of residual movements in patients with incomplete injuries.

Although the antispastic drugs shown in Fig. 5 can be problematic when taken orally, results from the studies summarized here open new possibilities for spinally directed approaches in controlling spasticity, such as the use of intrathecal drug delivery. First, cyproheptadine may provide better control of spasticity than baclofen because it works directly on the motoneuron, thereby preventing aberrant descending inputs from the cortex and brainstem from activating the motoneuron.

Fig. 5. Target sites for antispastic drugs. Presynaptic (1), motoneuron (2), and GABAergic (3) sites of action for antispastic drugs are depicted. Site 1: GABA_{b}, α_{2}, and 5-HT_{3} receptors (r) located on presynaptic sensory terminals or on pre- or postsynaptic sites on interposed excitatory interneurons activated by baclofen, tizanidine, and zolmitriptan, respectively, to reduce glutamate release and activation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors on motoneuron. Site 2: 5-HT_{2}/α_{1} receptors on motoneuron with constitutive or ligand activation, which facilitates downstream voltage-gated calcium channels (CaV) mediating PICs via G_{q} protein-coupled pathways. Inverse agonists switch the 5-HT_{2}/α_{1} receptors into their inactive states to reduce activity in the G_{q} pathway, lessen facilitation of CaV receptors, and reduce PICs and, consequently, muscle spasms. Site 3: spinal injection of the HIV1-CMV-GAD65 lentiviruses leads to an increase in GAD65 gene expression and GABA release from astrocytes. Combined systemic administration of tiagabine, a GABA reuptake inhibitor, increases levels of GABA to a sufficient level to activate pre- and postsynaptic GABA receptors to reduce spasticity. Cypro, cyproheptadine.
that baclofen does not affect. This approach may be useful for many causes of spasticity such as amyotrophic lateral sclerosis (ALS), cerebral palsy, and brain trauma/injury in addition to spinal cord injury. Second, intrathecal baclofen can have potentially fatal side effects if suddenly withdrawn, as occurs during sudden blockage of the catheter (Awaad et al. 2012; Lazarothes et al. 1990; Meythaler et al. 2003; Mohammed and Hussain 2004; Stempfen and Tsai 2000). Thus a potential strategy would be to give a combination of GABA<sub>B</sub>, α<sub>2</sub>, and 5-HT<sub>1B/1D/F</sub> receptor agonists, which all converge to activate G<sub>i</sub>-coupled pathways, at individually lower doses to potentially reduce severe side effects after sudden drug withdrawal.

Another spinally targeted strategy has recently been proposed by Marsala’s group (site 3, Fig. 5) whereby in a rat model of ischemic spinal cord injury, increases in GABA release and reduction of spastic stretch reflexes were produced by the combined upregulation of GAD65 gene expression in lumbar astrocytes (from spinal injections of lentivirus) and the systemic administration of tiagabine, a GABA uptake inhibitor (Kakinohana et al. 2012). With spinally targeted interventions (intrathecal or spinal transfections), spasticity may be better controlled without the unwanted side effects of sedation, tolerance, and appetite stimulation. The combined use of these strategies, including activating 5-HT<sub>1B/1D/F</sub> receptors and suppressing 5-HT<sub>2A</sub> receptor activity, provides new avenues for antispastic treatment.

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Disclosures

No conflicts of interest, financial or otherwise, are declared by the authors.

Author Contributions

J.D. and M.A.G. conception and design of research; J.D., Y.L., D.J.B., and M.A.G. performed experiments; J.D., Y.L., and D.J.B. analyzed data; J.D. and M.A.G. interpreted results of experiments; J.D., D.J.B., and M.A.G. prepared figures; J.D. drafted manuscript; J.D. and M.A.G. edited and revised manuscript; J.D., Y.L., D.J.B., and M.A.G. approved final version of manuscript.

References


D’Amico JM, Gorassini MA. Sensory afferent transmission to the motoneuron is reduced by 5HT<sub>1A</sub> receptor activation in uninjured and spinal cord injured subjects. Soc Neurosci Abstr 552.02, 2012.

D’Amico JM, Li C, Bennett DJ, Gorassini MA. Constitutively active 5HT<sub>1A</sub> receptors facilitate muscle spasms in human spinal cord injury. J Neurophysiol (December 5, 2012). doi:10.1152/jn.00821.2012.


M.A.G. performed experiments; J.D., Y.L., and D.J.B. analyzed data; J.D. and M.A.G. prepared figures; J.D. drafted manuscript; J.D. and M.A.G. edited and revised manuscript; J.D., Y.L., D.J.B., and M.A.G. approved final version of manuscript.

References


Martin GR. Pre-clinical pharmacology of zolmitriptan (Zomig; formerly 311C90), a centrally and peripherally acting 5HT1B/1D agonist for migraine. Cephalalgia 17, Suppl 18: 4–14, 1997.


