Reduction of Spinal Sensory Transmission by Facilitation of 5HT1B/D Receptors in Non-injured and Spinal Cord Injured Humans

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Activation of receptors by serotonin (5HT1) and noradrenaline (α2) on primary afferent terminals and excitatory interneurons reduces transmission in spinal sensory pathways. Loss or reduction of descending sources of serotonin and noradrenaline after spinal cord injury (SCI) and the subsequent reduction of 5HT1/α2-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. Here we test in a double-blind, placebo controlled study if facilitating 5HT1B/D receptors with the agonist zolmitriptan reduces the sensory activation of motoneurons during an H-reflex in both non-injured 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% (non-injured) and 62% (SCI) of pre-drug 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 post-stimulation to end of reflex) known to be mediated by persistent inward currents in the motoneuron. These results demonstrate that facilitation of 5HT1B/D receptors reduces sensory transmission in both monosynaptic and polysynaptic reflex pathways to ultimately reduce long-lasting reflexes (spasms) after spinal cord injury.
INTRODUCTION

After a complete spinal cord injury (SCI), levels of serotonin and noradrenaline below the lesion decrease greatly because the major supply of these neuromodulators comes from descending pathways originating in the brainstem (Anden et al. 1964; Carlsson et al. 1963; Jacobs et al. 2002; Jordan et al. 2008; Rekling et al. 2000). Serotonin and noradrenaline normally inhibit transmission of ascending and segmental sensory pathways via the activation of Gi-coupled serotonin (5HT1) and noradrenaline (α2) receptors located on sensory afferent terminals and excitatory interneurons (Clarke et al. 2002; Di Pasquale et al. 1997; Engberg et al. 1968; Jankowska et al. 1993; Jordan et al. 2008; Manuel et al. 1995; Millan 2002; Rekling et al. 2000; Singer et al. 1996; Yoshimura and Furue 2006). The reduction of 5HT1 and α2 receptor activation (Murray et al. 2011; Rank et al. 2011) results in the enhanced transmission of low-threshold, cutaneousmuscular afferent pathways to produce abnormally long (~1 s), excitatory postsynaptic potentials (EPSPs) in response to brief sensory stimulation in both rat and human motoneurons (Li et al. 2004a; Norton et al. 2008). These long polysynaptic EPSPs contribute to spasticity after SCI by triggering slowly activating, calcium persistent inward currents (CaPICs) that drive self-sustained motoneuron firing and involuntary muscle spasms (Bennett et al. 2001b; Gorassini et al. 2004; Heckman et al. 2008; Li et al. 2004a).

One strategy to reduce muscle spasticity after SCI has been to facilitate the activation of Gi-coupled pathways to reduce sensory transmission to motoneurons. The common anti-spastic medication baclofen, a GABAb-receptor agonist, and clonidine/tizanidine, α2-receptor agonists, activate Gi-coupled pathways to reduce cAMP and Ca++ entry into synaptic terminals (Curtis et al. 1997). This ultimately results in reducing the duration of polysynaptic EPSPs from about 1s to less than 50 ms and as a consequence, the CaPIC is not activated and muscle spasms are
reduced (Li et al. 2004b; Murray et al. 2011; Rank et al. 2011). However, both baclofen and
tizanidine produce undesirable side effects such as tolerance, sedation and hypotension (Davidoff
1985; Gracies et al. 1997; Krach 2001; Meleger 2006; Rosche 2002) and thus, are not optimal
oral anti-spastics. Recently, another strategy to activate Gi-coupled pathways via 5HT1 receptors
has been tried where both short- and long-latency polysynaptic EPSPs were reduced by either
5HT1B or 5HT1F receptor facilitation (Murray et al. 2011), leading to a reduction in CaPIC
activation and muscle spasms. Application of the various 5HT1B/F receptor agonists did not
reduce the CaPIC itself, demonstrating that their anti-spastic effects were mediated by reducing
the duration of sensory, synaptic activation of the motoneuron so that the slowly activating
CaPICs were not recruited.

One of the 5HT1 receptor agonists tested in the Murray et al. 2011 study was
zolmitriptan, a 5HT1B/D receptor agonist that is approved for human use to treat migraines
(Martin 1997; Martin et al. 1997; Peterlin and Rapoport 2007). Although zolmitriptan cannot be
used on a daily basis due to adverse side effects, and thus cannot be used as an anti-spastic, we
tested as a proof of principle if facilitation of 5HT1B/D receptors in humans also reduces
segmental sensory transmission to motoneurons in both non-injured control and SCI participants.
To examine the activation of the motoneuron by sensory inputs in isolation without CaPIC
activation, we examined the effect of zolmitriptan on the soleus monosynaptic H-reflex. The
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
estimated to be ≈30 ms in humans based on 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 if long-lasting, polysynaptic reflexes activated by cutaneomuscular stimulation to the medial arch of the foot, and the subsequent long-lasting reflexes (spasms) they trigger, were also reduced by zolmitriptan to determine if facilitation of 5HT1B/D receptors is a potential strategy to reduce spasticity after SCI. Parts of this 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 anti-migraine drug zolmitriptan, in non-injured and SCI participants, was approved by Health Canada-Clinical Trials. All participants gave written, informed consent prior to participating in the study. In total, 6 non-injured control (35 ± 13 yrs, 2 female) and 7 SCI participants with motor complete injuries (35 ± 10 yrs, 2 female) took part in the study (Table 1). Three of the 7 SCI participants (1-3M, Table 1) also took part in the 5HT2 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. A further 2 SCI participants were excluded from the study (both motor complete, T11/12 and T6/7) because appreciable H-reflexes could not be evoked from either leg.

Drug Administration

All participants were required to come to the lab on two separate occasions (separated by at least 1 week) 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. Non-injured control and SCI participants received a 10mg dose of the 5HTB/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 minutes after drug intake. Participants were asked to report any physiological
sensations after taking zolmitriptan or placebo. Because plasma concentrations of zolmitriptan
are detectable at 15 minutes after oral intake with peak concentrations occurring at 2-4 hours
(Peterlin and Rapoport 2007), reflex recordings were taken every 30 min for 2 hours after drug
intake. This allowed us to examine the onset of the drug affect and make measurements near the
time of peak plasma concentrations. In pilot experiments, taking reflex recordings beyond 2
hours after drug intake was too fatiguing for the participants.

H-reflex Recordings

H-reflex recordings were obtained in both non-injured 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 as they could not produce voluntary contractions. All non-injured
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 (1M and 4F in Table 1). Two surface electrodes (2.2 x 3.3 cm, Kendall Soft-
E, Chicopee, MA, USA) were placed over the right soleus muscle to record EMG signals. The
soleus H-reflex was evoked by stimulating the tibial nerve (DS7A constant current stimulator
NL703, Digitimer, Hertfordshire, UK) through a monopolar electrode once the best position was
found with a probe electrode (1ms pulse width, return electrode placed over patella). The
surface EMG signal was amplified 200 or 1000 times (depending on the size of the response) and
filtered using a bandpass of 20-2500Hz (Model 2024F Intronix Technologies, Bolton, ON,
Canada). All signals were digitized at a rate of 5kHz using Axoscope hardware and software (Digidata 1440 Series, Molecular Devices, Sunnyvale, USA) and stored on a personal computer for offline 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 minutes after drug/placebo intake. Prior to each H-reflex recruitment curve, the motor threshold (MT) was determined online as the stimulation intensity required to elicit an M-wave of approximately 100µV. The stimulation intensity was expressed as a multiple of motor threshold (xMT) and was set from below H-reflex threshold (ranging from 0.5 to 0.7 xMT) to when the H-reflex decreased after its peak (ranging from 1.2 to 1.6 xMT) in steps of 0.05 xMT. This ensured that a minimum of 8-9 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 3s which allowed enough time to manually increase the stimulation intensity after every 5th trial. The maximal motor response (Mmax) 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 Mmax. The amplitude of the normalized H and M-waves were plotted at each stimulation intensity, the later expressed as a function of motor threshold (MT), to produce H and M-wave recruitment curves. To standardize the measurement of MT across the different time points and participants, MT was re-
calculated 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 3-parameter sigmoid function \[H(s) = \frac{H_{\text{max}}}{1+e^{m(S50-s)}}\] (Klimstra and Zehr 2008). The peak H-reflex (H_{\text{max}}) and the stimulation intensity producing 50% of the H_{\text{max}} (S50) were measured off the fitted curve. The slope parameter “m” was too variable because it depended on the number of points along the recruitment curve and therefore, 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 non-injured controls and from 0.87 to 0.99 (median = 0.98) in SCI participants. The threshold to evoke an H-reflex (H_{\text{thresh}}) was measured as the stimulus intensity required to elicit an H-reflex that was 5% of H_{\text{max}}. Because there was large variability in the size of H-reflexes between SCI participants, the 3 parameters of the H-reflex recruitment curve (H_{\text{max}}, S50 and H_{\text{thresh}}) at the 30, 60, 90 and 120 minute time points were expressed as a percentage of the pre-drug value and averaged across subjects.

**Cutaneomuscular Reflex Recordings**

Cutaneomuscular reflexes were recorded in SCI participants only because long-lasting responses (> 1s) cannot be evoked in non-injured control participants. Cutaneomuscular 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
Cutaneomuscular afferents supplying the side and sole of the foot were stimulated with long pulse trains applied to the medial arch of the foot (300Hz, 14 pulses, 0.5ms pulse width; DS7A constant current stimulator) at an intensity that was just below pain threshold (40±15mA on average). Surface EMG signals from the TA were amplified 1000 times and filtered using a band-pass of 20-2500Hz (Model 2024F Intronix Technologies, Bolton, ON, Canada). Both limbs were tested and the TA muscle that exhibited the longest reflex response pre-drug 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 seconds for each trial. Three to four pre-drug reflex responses were recorded until two consecutive responses fell within 10% of each other. These last two pre-drug reflex responses were averaged together to form the baseline reflex response. Cutaneomuscular reflex recordings were repeated at 30, 60, 90 and 120 minutes after drug intake and were performed immediately after each H/M recruitment curve.

Cutaneomuscular Reflex Analysis

The cutaneomuscular 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++- channel blocker isradipine (Li et al. 2004a; Murray et al. 2011). The average latency of the LPR was 84±14ms, with an average duration of 215±15ms. The later, long-lasting reflex component (LLR) was defined as the time window from 500ms after the first stimulation pulse to the end of the reflex response in the pre-
drug trial, as per Murray et al. 2010, 2011 and Rank et al. 2011 (LLR duration:400±158ms).

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 300ms post stimulation, LLR: 500ms post-stimulation to end of pre-drug response). The mean rectified background noise, measured from 100ms before the stimulation, was subtracted from the data. The mean EMG for each of the 6 sweeps were averaged together to obtain LPR and LLR values for each time point. All values were expressed as a percentage of the pre-drug 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⁻¹), 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⁻¹); recordings were made starting 2.5 hr later, as detailed previously (Bennett et al. 2001a; Li and Bennett 2003). Ventral (S4 and C01, coccygeal) and dorsal (C01) 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: ≈3x’s sensory afferent threshold; repeated 5x’s
every 10s for one trial, trials were repeated every 12 minutes). With this stimulation, a mono-
synaptic reflex with a latency of 2 ms and lasting for $\approx 4$ ms was evoked in the ventral roots. A
300nM 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 software. Values in the text are
expressed as mean ± standard deviation and in the graphs as mean ± standard error. Normality
for the parameters of the H-reflex recruitment curve (Hmax, S50 and Hthresh) and for the LPR
and LLR components of the cutaneomuscular 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 Square
test) for non-normally distributed data was used to determine if there was an effect of the drug on
the reflex parameters over the 30, 60, 90 and 120 minute 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-HT1B/D receptor agonist zolmitriptan reduced the amplitude of the maximum H-reflex (Hmax) in both non-injured and spinal cord injured participants. The peak-to-peak amplitude of Hmax was reduced 120 minutes after zolmitriptan intake at similar stimulation intensities to pre-drug as reflected in the matched M-wave before (black trace) and after (gray trace) drug intake for both non-injured control (Fig. 1A) and SCI (Fig. 1C) participants. As shown for the participants in Fig. 1, the average unnormalized Hmax measured before zolmitriptan intake was significantly larger in controls (2.95±1.36mV) compared to SCI participants (1.35±1.31mV, p=0.05). Likewise, Mmax was larger in controls (6.00±2.50mV) compared to SCI (3.10±1.58mV, p=0.03), resulting in Hmax/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 (Figs. 1B&D), zolmitriptan mainly affected the amplitude of the H-reflex and not its overall excitability as there were no lateral shifts in the recruitment curve plotted as a function of motor threshold (MT). The reduction in H-reflex size occurred even thought 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 minutes after drug intake (triangles). In 4/6 non-injured controls and in 5/7 of the SCI participants, the H-reflex was suppressed at all stimulation intensities, as shown for the two participants in Figures 1B&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
minutes post-drug (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).

Insert Figure 1 near here

Group Data: H-reflex recruitment curve

When plotting the normalized Hmax as a percentage of the pre-drug value across the
different time points, Hmax after zolmitriptan intake (solid circles) deviated from placebo values
(open circles) at 60 minutes and onwards, with Hmax being reduced to 67.5 ± 0.27% and 70.6 ±
0.21% of pre-drug values at 120 minutes in non-injured controls (Fig. 2A) and SCI participants
(Fig. 2B) respectively. There was a significant reduction in Hmax over time after zolmitriptan
intake compared to pre-drug (controls: F= 4.77, p= 0.007; SCI: F= 3.318, p=0.027) but not after
placebo (controls: F= 0.62, p=0.65; SCI: F=0.42, p=0.79). Two-way ANOVA revealed a
significant drug x time interaction (F=3.507, p=0.025), with post-hoc tests showing Hmax after
zolmitriptan intake was significantly smaller compared to placebo at the 60, 90 and 120 minutes
time points (all p < 0.05). There were no significant increases in Mmax over time (expressed as
% of pre-drug, Figs. 2A&B bottom graphs) after either placebo or zolmitriptan in both controls
and SCI participants (all F and Chi squares > 0.55 and 1.86 respectively, all p > 0.33), indicating
that the observed decreases in the normalized Hmax did not result from dividing Hmax by a
steadily increasing Mmax.
In some subjects, the peak reduction in $H_{\text{max}}$ did not occur at 120 minutes after zolmitriptan intake but earlier at 90 minutes ($n = 3$ control, $n = 1$ SCI) or 60 minutes ($n = 1$ control, $n = 1$ SCI). Thus, when plotting the peak decrease in $H_{\text{max}}$ occurring at either of these time points (60, 90 or 120 minutes), the $H_{\text{max}}$ as a percentage of pre-drug was even lower at $58.9 \pm 0.1\%$ for controls and $62.3 \pm 0.23\%$ for SCI participants (Fig. 2C), with the peak decrease in $H_{\text{max}}$ similar between the two groups ($p = 0.78$). In 3 preliminary control participants, the dosage of zolmitriptan needed to be at least 10 mg to see a reduction in $H_{\text{max}}$ given that 5mg, like placebo, did not produce a decrease in $H_{\text{max}}$ (Fig. 2D). Finally, as reflected in the recruitment curves of Figure 1, there were no changes in $H_{\text{thresh}}$ or $S_{50}$ for non-injured control and SCI participants after zolmitriptan or placebo (all $F > 0.16$, all $p > 0.12$).

Insert Figure 2 near here

**Cutaneomuscular Reflex in SCI**

Long-duration reflex responses (spasms) were evoked in the tibialis anterior (TA) muscle in response to a train of pulses (300Hz, 14 pulses, 0.5ms pulse width) applied to the medial arch of the foot as shown for the two participants in Figures 3A&B. Both the long polysynaptic component of the reflex (LPR, marked by gray bar), which is mediated by both sensory-evoked EPSP’s and PICs, and the long lasting reflex component (LLR, marked by black bar), which is mainly mediated by PICs (see **Cutaneomuscular Reflex Analysis** in Methods for rationale) were reduced by zolmitriptan. It was possible to evoke long-duration reflexes in 6 of the 7 motor complete SCI participants. Similar to the H-reflex, zolmitriptan reduced the size of the LPR over time (Fig. 3C), decreasing it to $46.1 \pm 0.32\%$ of pre-drug values at 120 minutes ($F=$
8.92, p<0.001). In comparison, there was no decrease of the LPR after placebo intake (Chi square=7.07, p=0.13). A two-way ANOVA revealed a significant drug x time interaction (F=5.325, p=0.004), with the LPR after zolmitriptan significantly smaller compared to placebo at the 60, 90 and 120 minute 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 square=3.68, p=0.45), with the LLR being reduced to 25.0 ± 0.39% of its pre-drug value at 120 minutes. Two-way ANOVA revealed a significant drug x time interaction (F=3.67, p=0.026), with the LLR significantly smaller after zolmitriptan compared to placebo at the 30, 60, 90 and 120 minute 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 5HT1B/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 300nM of 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 5 out of 5 rats tested at 15 minutes after bath application of the drug.

Short-latency polysynaptic reflexes: rat and human

In some rats, rather than a monosynaptic reflex, a short-latency polysynaptic reflex (SPR) was evoked in the ventral root which lasted from 10 to 40ms post-stimulation (Fig. 4C) and that was also reduced by zolmitriptan (Fig. 4D from Murray et al. 2011). A similar distinct SPR was also evoked in 3 of the SCI participants during the cutaneomuscular reflex recordings (see also D’Amico et al. 2012). The SPR had a latency of \( \approx 70 \)ms and a duration of 50ms (Fig. 4E). In all 3 SCI participants, zolmitriptan reduced the SPR to 56% of its pre-drug value 120 minutes after drug intake, as shown for the SCI participant in Figure 4F (6M, Table 1).
DISCUSSION

We demonstrated that facilitation of 5HT1B/D receptors with zolmitriptan, but not placebo, reduced sensory transmission to motoneurons as evidenced by the suppression of H-reflexes in non-injured and spinal cord injured 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 5HT1B/D receptor agonists may be useful to control sensory transmission and reduce the triggering of muscle spasms after spinal cord injury.

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 5HT1B/D receptor facilitation. In the remainder of participants (5/13), only H-reflexes activated at 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 Mwave activation, the later an indirect indication that the number of sensory afferents activated pre and post-drug was similar (Misiaszek 2003; Zehr 2002). The decrease in Hmax and the absence of any change in Hthresh or S50 suggests that facilitation of 5HT1B/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
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 pre-synaptic inhibition on terminals of sensory afferents or excitatory interneurons or from post-synaptic inhibition of excitatory interneurons, as a result of 5HTB/D receptor facilitation. In addition, because H-reflexes were evoked at a rate of 0.33 Hz, a frequency where “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 spinal cord injury. Reduction of the polysynaptic reflexes was also associated with a reduction in long-lasting reflexes (LLR or spasms). As shown from animal studies with very similar cutaneous reflexes to humans, the LPR can last for 500-1000ms and is a ≈50% mixture of EPSP and PIC activation whereas the LLR, 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 LLR (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 5HT1B/D-receptor facilitation.

**Effect of Zolmitriptan on sensory transmission likely via 5HT1B/D receptors**

Zolmitriptan is a commonly prescribed anti-migraine medication that is able to cross the blood-brain barrier, though only at relatively high doses (Proietti-Ceccini et al. 1997; Visser et al. 1996; Werhahn et al. 1998). It displays high affinity to 5HT1B (Ki=5.01nM) and 5HT1D (Ki=0.63nM) receptors with modest affinity to the 5HT1F receptor (Ki=63.09nM)(Martin et al. 1997). Zolmitriptan likely exerts at least part of its effects on the transmission of cutaneous afferent pathways via activation of the 5HT1B receptor given that in animal studies, the potency of various 5HT1 receptor agonists in reducing cutaneous polysynaptic reflexes and sensory-evoked EPSPs was correlated to the published binding affinity of only 5HT1B and 5HT1F receptor agonists and not to other 5HT receptor agonists (Murray et. al. 2011). Likewise, only 5HT1B-receptor antagonists reversed the effects of zolmitriptan on long-latency polysynaptic reflexes in animals. Thus, the activation of 5HT1B, and not 5HT1D receptors, by zolimitriptan likely produced the reduction of cutaneous 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 5HT1B receptor or if there is also involvement of the 5HT1D receptor that
is also facilitated by zolmitriptan (Honda et al. 2003).

It is interesting that zolmitriptan produced similar decreases in H-reflexes in participants
with and without SCI at the 10mg dose, even though reduced levels of endogenous 5HT were
likely present below the lesion in the SCI participants (Murray et al. 2010). This suggests that
after SCI, 5HT1B/D receptors do not develop supersensitivity to applied agonists, similar to
findings in rats where intravenous administration of sumatriptan, a similar 5HT1B/D receptor
agonist, depressed the monosynaptic reflex to the same degree in non-injured and spinal cord
injured rats (Honda et al. 2006). It would be interesting to examine if other 5HT receptors, such
as 5HT1A, 5HT2A and 5HT7 receptors, which have been shown to help facilitate locomotion
after spinal cord injury (Antri et al. 2005; Vinay et al. 2012), develop supersensitivity to 5HT
receptor agonists in the presence of reduced levels of endogenous 5HT.

Clinical Implications

Following spinal cord injury, the activation of Gq-coupled pathways in the motoneuron
via constitutive 5HT2/α1 receptors facilities 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 Figure 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 manuscripts. One strategy to reduce muscle spasms is to
reduce 5HT2/α1 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 anti-spastic drugs used currently (discussed below). Thus, the
suppression of 5HT2/α1 receptors, specifically 5HT2B/C and α1A 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 in patients
where 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 5HT2B/C and α1A
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 as 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 Gi-coupled
pathways (site 1, Fig. 5) with GABAβ- (baclofen, Curtis et al. 1997; Li et al. 2004b), α2-
(tizanidine, Krach 2011; Meleger 2006) and, based on the current study, 5HT1B/D-
(zolmitriptan) receptor activation. The main anti-spastic effect of these drugs is to reduce the
sensory-evoked EPSP from direct afferent and interposed interneuronal inputs, allowing an
unmasking of an 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 Gi-
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 byproduct production and ironically, induction of headaches (Martin 1997; Peterlin and Rapoport 2007). Again, this study highlights the need to develop better 5HT1B/D, and possibly 5HT1F 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 anti-spastic drugs shown in Figure 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 that baclofen does not effect. This approach may be useful for many causes of spasticity such as 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; Lazorthes et al. 1990; Meythaler et al. 2003; Mohammed and Hussain 2004; Stempien and Tsai 2000). Thus, a potential strategy would be to give a combination of GABAb-, a2- and 5HT1B/D/F- receptor agonists, which all converge to activate Gi-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 5HT1B/D/F receptors and suppressing 5HT2/α1 receptor activity, provides new avenues for antispastic treatment.
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Martin GR. Pre-clinical pharmacology of zolmitriptan (Zomig; formerly 311C90), a centrally and peripherally acting 5HT1B/1D agonist for migraine. *Cephalagia* 18: 4-14, 1997.


Figure Legends

**Figure 1: H-reflexes after Zolmitriptan in Uninjured Control and SCI Participant**

**A** M-wave (M) and maximum H-reflex (Hmax) recorded in a single uninjured control participant before (black trace) and 120 minutes after (gray trace) 10 mg of zolmitriptan. **B** Corresponding H-reflex and M-wave recruitment curve from same participant in A plotted as peak-to-peak and normalized to Mmax. Stimulation intensity expressed as a multiple of motor threshold. The two pre-drug H-wave recruitment curves (Pre1&2) are represented by black lines and solid circles, the 30 min curve by a dark gray line and open circles, the 90 min curve by a light gray line and solid triangles and the 120 min curve by a dark gray line and open triangles (60 minute data not shown for clarity). M-wave recruitment curves have similar line color schemes with no symbols for clarity. **C** and **D** similar to (A) and (B) but for a T3-4 SCI participant (2M in Table1).

**Figure 2: Group Data: Hmax and Mmax after Zolmitriptan**

**A** Top panel: Averaged Hmax, expressed as a percentage of pre-drug, at 30, 60, 90, and 120 minutes after zolmitriptan (open circles) and placebo (Plb, solid circles) intake in 6 uninjured control participants. Bottom panel: Average Mmax expressed as a percentage of pre-drug at all time points after zolmitriptan (open circles) and placebo (solid circles). **B** Same as in (A) but for averaged data across the 7 SCI participants. **C** Peak decrease in Hmax (expressed as % of Pre-drug) irrespective of time after zolmitriptan intake for both uninjured control (open bar) and SCI (solid bar) participants. **D** Averaged Hmax (expressed as % of Pre-drug) for 3 uninjured control participants receiving placebo (black line), 5mg (gray line) and 10mg (open circle) of zolmitriptan. Error bars in this and following graphs represent mean ± standard error. * p < 0.05, ** < 0.01, *** < 0.005.
Figure 3: Zolmitriptan and CMR in SCI Participants  

A) Overlay of 6 unrectified TA EMG traces before (upper traces) and after 10mg zolmitriptan (bottom traces) in single C6-7 SCI participant (5M, Table1). Grey bar denotes the window calculated for the long polysynaptic reflex (LPR, start of reflex to 300ms), and the black bar denotes the long-lasting reflex (LLR, 500ms post stimulation to end of reflex).  

B) Similar to A but for C6-7/L3 SCI participant (4F, Table1).  

C) Averaged LPR, expressed as a % of Pre-drug at 30, 60, 90 and 120 minutes after 10 mg zolmitriptan (open circles) and placebo (open circles) in 6 of the 7 SCI participants (1M-6M, Table 1).  

D) Same as in C but for the LLR in 5 of the 7 SCI participants (1M-5M, Table1).  

Figure 4: Effects of Zolmitriptan Verified in 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 300nM bath application of zolmitriptan.  

C) Short-latency polysynaptic reflex (SPR) recorded from a ventral root of a different chronically injured rat (no monosynaptic response was evoked).  

D) Polysynaptic reflex from C after 300nM application of zolmitriptan (modified from Fig. 5 in Murray et al. 2011).  

E) Overlay of 6 SPR’s recorded from tibialis anterior in spinal cord injured participant (3M, Table 1) before and F) 120 minutes after 10mg of oral zolmitriptan. In all figures asterisks mark time of single pulse, or start of multiple pulse, stimulation.  

Figure 5: Target Sites for Anti-spastic Drugs  

Pre-synaptic (1), motoneuron (2) and GABAergic (3) sites of action for anti-spastic drugs. Site 1: Gamma-Aminobutyric Acid (GABA)b- , α2- and 5HT1- receptors 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 $\alpha$-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and N-methyl-D-aspartate (NMDA) receptors on motoneuron. Site 2: 5HT2/$\alpha_1$-receptors on motoneuron with constitutive or ligand activation which facilitates downstream voltage-gated calcium channels (CaV) mediating PICs via Gq-protein coupled pathways. Inverse agonists switch the 5HT2/$\alpha_1$-receptors into their inactive state to reduce activity in the Gq pathway, lessen facilitation of CaV receptors, reduce PICs and consequently, muscle spasms.

Site 3: Spinal injection of the HIV1-CMV-GAD65 lentivirus 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.
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**Table 1: SCI participants.** Demographics of SCI participants including age of participant and their injury at time of experiment, injury level, ASIA Impairment Scale (AIS), cause of injury, Modified Ashworth Score (MAS) and Penn Spasm Frequency score. Last two columns describe spared light touch and pinprick sensation of lower leg. ✓ indicates preserved sensation from tested area of knee downwards.
Figure 1
Figure 2
Figure 3
Figure 4
Motoneuron

5HT2/α1r

Astrocyte

GAD65

CaV

GABAr

AMPAR/NMDAR

Interneuron

GABAbr

Sensory afferent

GABA

Cypro

Glutamate

Baclofen

Tiagabine

Tizanidine

Zolmitriptan

Calcium ions

Figure 5