

GABAPENTIN SUPPRESSES SPASTICITY IN THE SPINAL CORD-INJURED RAT

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Abstract—Spasticity poses a major detrimental impact on the quality of life in a significant number of people with spinal cord injury (SCI). Recent observations in our laboratory suggest that spinal transection at the sacral S₂ level induces a significant increase in glutamatergic input to sacrocaudal motoneurons during the time spasticity is present in the tail muscles. The present study examined the efficacy of gabapentin, an agent that has been shown to reduce glutamate release, in managing spasticity within the tail musculature.

Method: In this blinded, crossover study adult Sprague-Dawley rats with S₂ spinal transections were tested behaviorally for the progression of spasticity in the tail musculature using our established system. When the animals demonstrated a significant level of spastic behavior (e.g. increased response to quick stretch, noxious and non-noxious cutaneous stimuli), they received either saline or the antiepileptic agent gabapentin (GBP; 50 mg/kg i.p.) and were assessed behaviorally and electrophysiologically at 1, 3, 6, 12 and 24 h post-injection.

Results: Both spastic behavior and electromyography (EMG) activity were significantly decreased at 1 and 3 h post-GBP injection when compared with the activity level following administration of saline. Spastic behavior and EMG activity gradually increased over time and returned to baseline activity by 24 h post-injection.

Conclusion: Gabapentin diminishes both the behavioral and electrophysiological manifestation of SCI-induced spasticity, in the tail musculature, in a time dependent manner. © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.

Key words: muscle hyperreflexia, presynaptic glutamate inhibition.

Spasticity can be characterized as a “disordered sensorimotor control, resulting from an upper motor neuron lesion, presenting as intermittent or sustained involuntary activation of muscles” (Burrige et al., 2005; Pandyan et al., 2005; Platz et al., 2005; Wood et al., 2005). Spasticity has been reported in as many as 50–70% of spinal cord-injured (SCI) individuals (Kirshblum, 1999; Sköld et al., 1999; Walter et al., 2002), and can dramatically reduce the

person’s ability to participate in the activities of daily living that contribute to their well-being (Mayer, 1997; Proulx et al., 2000; Walter et al., 2002).

Currently the pharmacologic agent of choice for the treatment of SCI-induced spasticity is baclofen (a GABA_B receptor agonist). While continuous intrathecal infusion of baclofen can be effective in the treatment of severe spasticity, concern for baclofen tolerance in many individuals remains an issue (Soni et al., 2003). Additionally, baclofen has multiple side-effects and is not tolerated by all patients (Abbruzzee, 2002; Elovic, 2001; Kirshblum, 1999; Taricco et al., 2000).

We recently demonstrated that following S₂ spinal transection, in the rat, there is a significant increase in the number of vesicular glutamate transporter 2 (VGLUT2) labeled terminals contacting sacrocaudal motoneurons (Kitzman, 2006). VGLUT2 is a marker for glutamatergic inputs arising from spinal segmental (interneuronal) sources (Alvarez et al., 2004; Landry et al., 2004; Oliveira et al., 2003; Todd et al., 2003). This increase in VGLUT2 labeling was especially evident when the animals displayed significant spasticity in the tail musculature. Thus, instead of increasing the level of CNS inhibition with pharmacologic agents (e.g. baclofen or benzodiazepine), suppression of spastic behavior may also be accomplished by decreasing the level of glutamate released from presynaptic terminals.

One compound that has been shown to interfere with glutamatergic transmission and is safe for clinical use is gabapentin (GBP). GBP (Neurontin[®]; Pfizer, New York, NY, USA) is approved for the treatment of epilepsy and is widely used off-label for the treatment of neuropathic pain (Crawford et al., 1987; Levendoğlu et al., 2004; Morello et al., 1999; Rowbotham et al., 1998). Although GBP possesses multiple cellular mechanisms, recent work has suggested that inhibition of glutamatergic transmission may be preeminent in mediating its therapeutic effects in epilepsy, neuropathic pain, and perhaps spasticity (Wheeler, 2002). Specifically, GBP has been shown to inhibit presynaptic glutamate release (Coderre et al., 2005, 2007; Maneuf and McKnight, 2001; Maneuf et al., 2004; Shimoyama et al., 2000). When incorporated as an adjunct to standard pharmacological interventions for spasticity, GBP demonstrates the potential to help decrease the manifestation of spasticity in SCI-individuals (Gruenthal et al., 1997; Priebe et al., 1997). However, to date there has been no systematic examination of the efficacy of GBP as a single treatment for the management of SCI-induced spasticity.

The aim of the present study was to test the therapeutic efficacy of GBP, as a single treatment, in the management of SCI-induced spasticity in the tail musculature. We

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Abbreviations: ANOVA, analysis of variance; C-GBP, control animal receiving gabapentin; C-S, control animal receiving saline; EMG, electromyography/electromyographic; GBP, gabapentin; MA, maximal amplitude; RMS, root mean square; SCI, spinal cord injury/injured; SCI-GBP, spinal cord injured animal receiving gabapentin; PRE, pre-solution; SCI-S, spinal cord-injured animal receiving saline; VGLUT2, vesicular glutamate transporter 2; % MA, percent of the maximal amplitude.

hypothesize both the behavioral and electrophysiological responses will significantly differ between SCI spastic animals who receive GBP versus vehicle. A secondary hypothesis of this study was that GBP would not have a significant effect on either behavioral or electrophysiological responses in the animals with an intact nervous system.

EXPERIMENTAL PROCEDURES

Data were obtained from female adult Sprague–Dawley rats weighing 200–250 g. The care and handling of the animals were in accordance with institutional guidelines and have been approved by the Animal Care and Use Committee at the University of Kentucky. All procedures were carried out in accordance with the National Institutes of Health and Institutional Animal Care and Use Guidelines.

S₂ spinal transection model

Low sacral spinal cord transection, in both the feline and rodent, has been shown to be a reliable and reproducible model of spasticity development in the tail muscles, which are continuous with and analogous to the axial musculature. Spasticity in the tail musculature demonstrates many of the same characteristics found in the human spastic syndrome (Bennett et al., 1999, 2004; Kitzman, 2005, 2006; Ritz et al., 1992). These include increased muscle tone, hyperreflexia both in response to muscle stretch and cutaneous (noxious and non-noxious) stimulation, clonus, flexor spasms, and paraesthesia. In addition, spastic tail muscles demonstrate a significantly increased amplitude and duration of involuntary muscle activity in response to cutaneous stimulation when compared with non-injured animals. Thus, the S₂ transection model provides an excellent system for examining relatively long lasting events, which may represent a more clinically relevant time frame (e.g. a >5 s involuntary activation of a muscle would most likely pose a restriction on mobility and function).

Surgical procedures

Animals were deeply anesthetized with a combination of ketamine (80 mg/kg) and xylazine (10 mg/kg) and a laminectomy was performed at the L_{1–2} vertebral level. The dura was opened transversely and the spinal cords were completely transected at the S₂ level using fine forceps and iridectomy scissors. The S₂ root was identified based on its entrance into the cord at the rostral half of the L₂ vertebrae (Hebel and Stromberg, 1976). A second group of animals underwent a laminectomy-only surgery and served as an age-matched control group. A final group of animals did not undergo any surgery and served as a second age-matched control group. All animals were closely monitored for signs of distress or discomfort as per IACUC guidelines. Animals were at least 4 weeks post-injury prior to initiation of pharmacological testing.

Therapeutic administration of GBP

In this crossover, placebo-controlled study, all animals were administered vehicle (0.9% saline) only or vehicle plus GBP (TOCRIS, Ellisville, MO, USA). The primary investigator was blinded as to the make-up of each solution during testing. GBP was administered in a 50 mg/kg i.p. dose based upon the well-characterized effects of this dose level in rodent models of neuropathic pain and epilepsy (De Vry et al., 2004; Hofmann et al., 2003). Prior to administration of the test solutions all animals were assessed for baseline behavior and electromyographic (EMG) activity. The animals were administered the test solutions once a day for four consecutive days. On day 3 the animals were assessed at 1, 3, 6, 12 and 24 h post-injection for changes in behavioral activity of the tail musculature (see below). Following the 24 h post-injection

behavioral assessment, animals were administered the 4th dose and changes in EMG activity were assessed at 1, 3, 6, 12, and 24 h post-injection. Following a 2-week washout period the animals were again behaviorally and electrophysiologically assessed to determine baseline level of activity; followed by four daily injections of the solution not received during the 1st trial and assessment for changes in behavior and EMG activity in the tail muscles.

Behavioral assessment

Tail manipulations were performed with the animals in a Broome rat restraint in which the tail hangs out the back and is free to move over its full length. The progression of spasticity in the rat tail musculature following S₂ transection was described by Bennett et al. (1999) and all tests are routinely performed in our laboratory (Kitzman, 2005, 2006, 2007). In the present study all animals displayed a stage-3 or stage-4 level of spasticity prior to initiation of the pharmacological intervention. Briefly, *stage-3* spasticity was characterized by: 1) flexion of the tail of >180° following quick stretch of the distal tail that lasts >10 s; repeated stretching increases the flexion response. This is in contrast to normal (un-injured) animals in which quick stretch of the distal tail elicits minimal flexion (<45°) that lasts for 2–3 s then returns to neutral. 2) Pronounced withdrawal of the tail in response to light touch (non-noxious stimulus), 3) light pinch (noxious stimulus) of the distal tail produces a curling >180° that is maintained for >5 s are in contrast to normal animals in which the tail curls 45–90° and returns to neutral position within 2–3 s following the pinch. 4) Clonus of the tail musculature may be present. *Stage-4* spasticity was characterized by: 1) an increased response of both tail flexor and extensor muscles to quick stretch, which is demonstrated by the tail assuming a coiled or S-shape lasting >10 s. In addition, the tails in a large percentage of animals frequently demonstrate a writhing type of motion in which there was an alternation between right–left muscle (flexor, extensor and abductor) activation lasting >2–3 s following the stretch. 2) Light pinch also produces coiling and/or writhing of the tail musculature. 3) clonus is present, and 4) spontaneous spasms of both tail flexor and extensor muscles can be present. For consistency a single experienced observer performed all behavior assessments. For the purpose of statistical analysis, responses to quick stretch, pinch, and mechanical flick were graded using a 5-point scale in which 0= minimal (≤45° flexion) response to the stimulus, 1=50–90° flexion, 2=>90°–180° flexion, 3=>180°–225° flexion, 4=>225°–360° flexion, and 5=significant coiling of the tail and/or activation of flexors, extensors, and abductors (writhing) lasting >2 s and the presence of clonus. The response to light touch was scored using a 3-point grading scale in which 0=no response, 1=minimal flexion of the tail away from the stimulus, and 2=pronounced flexing of the tail away from the stimulus.

Electrophysiological recording

The EMG data were recorded in awake animals using the BIOPAC MP150 System (BIOPAC Systems, Inc., Goleta, CA, USA). Two 4 mm shielded surface electrodes were used to collect data from the tail musculature. The ventral surface of the tail was abraded and cleaned with alcohol prior to electrode placement. The ground electrode was placed at midtail, the active (recording) electrode was placed 15 mm rostral to the ground, and the reference electrode was placed 10 mm caudal to the ground. Four 40 s trials were recorded at each time point. A pinch (noxious cutaneous) stimulus was applied following the first 10 s of each trial and the response EMG activity was then collected for the subsequent 30 s. The pinch was applied approximately 2.54 cm from the tip of the tail and was applied at 6.0±0.38 kg of force. The same investigator applied the pinch stimulus for all trials and all animals. Care was taken not to cause bruising in the area in which the stimulus was being applied.

EMG data reduction

EMG data were collected with a gain set at 2000 and the raw data were band pass filtered from 1 Hz–5 kHz. The raw EMG data were processed using Datapac software (Run Technologies, Mission Viejo, CA, USA). A passive demeaning filter was used to remove the DC offset. The mechanical (pinch) noxious stimulation point was defined as 3 standard deviations above the baseline reference interval with a minimal duration of at least 40 ms. The computer algorithm defined onset and offset of the mechanical stimulus. To assure the algorithm defined only the stimulus, a single investigator visually confirmed each trial. EMG data were normalized to 100% maximal amplitude (MA) for comparison across the time points and prior to administration of either vehicle or GBP (Benoit et al., 2003; Burden and Bartlett, 1999; Burden et al., 2003). The root mean square (RMS) amplitudes of 100 ms of

muscle activity prior to pinch stimulus served as the zero offset, while the greatest RMS amplitude of 500 ms time window following the pinch stimulus served as MA. The mean of the four MA that occurred represented 100%.

The response to the noxious stimuli over time was of interest due to its potential clinical relevance of restricting mobility. A momentary maximal event of electrical activity is not necessarily adequate to cause a clinical restriction of motion; therefore, we examined the electrophysiological response for a longer time frame. A greater activation of musculature for a sustained duration would implicate a more reactive response. During preliminary data analysis it was determined that in SCI spastic animals 6 s post-stimulus represented the typical time frame required for the increased EMG amplitude to return to baseline (Fig. 1). Elevated muscular activity for this duration would impact functional activity.

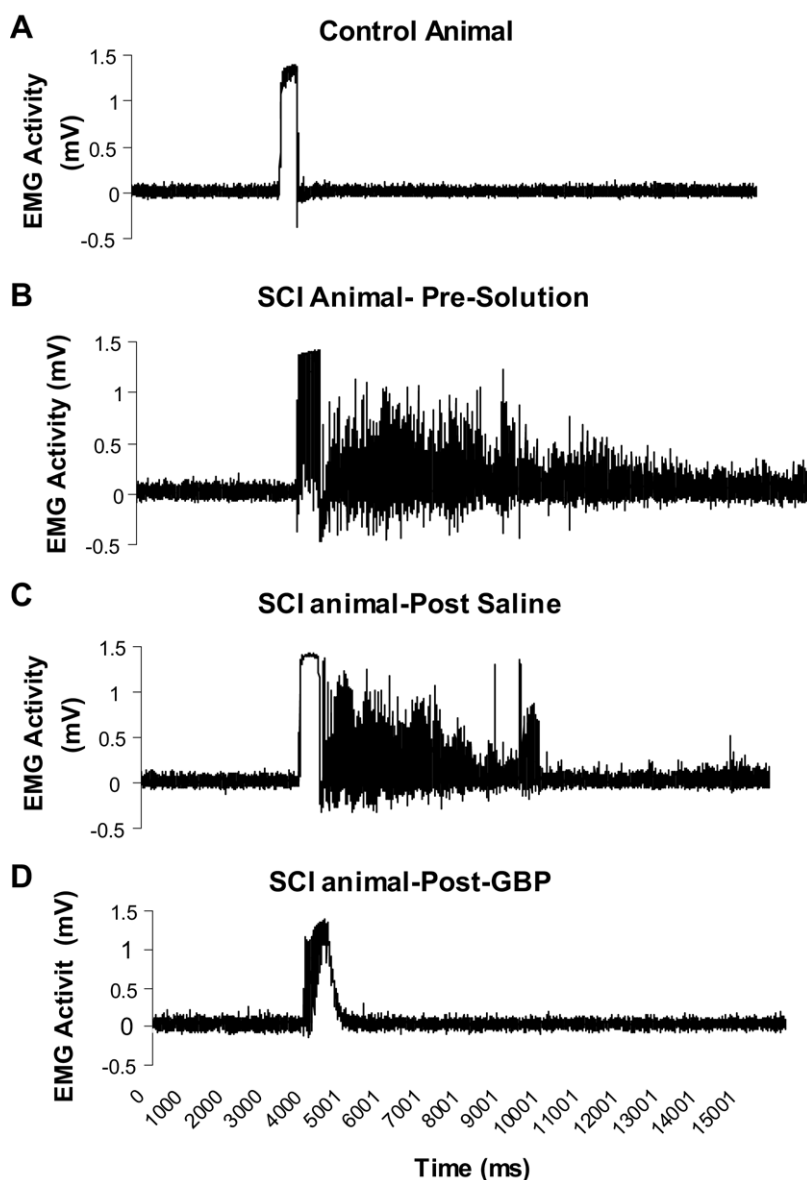


Fig. 1. EMG activity in the tail musculature. Fig. 1A demonstrates the EMG response to a pinch stimulus in a control (non-SCI) animal. B–D demonstrates the EMG responses to a pinch stimulus in the same SCI spastic animal prior to administration of any drug (Fig. 1B), 1 h following administration of saline (Fig. 1C), and 1 h following administration of GBP (Fig. 1D). Notice EMG activity significantly decreased in the spastic animals 1 h post-administration of GBP (50 mg/kg).

Thus, the EMG response measured every 500 ms over the first 6 s post pinch-stimulus was averaged and reported as percent of the maximal amplitude (% MA). This dependent measure represented the electrophysiological response to noxious stimulus.

Statistical analysis

Behavioral assessment. To determine if behavioral responses differed across time conditions for each solution, separate Kruskal-Wallis (nonparametric analysis of variance (ANOVA)) tests were performed. Non-parametric analysis techniques were employed due to the semi-quantitative nature of the outcome variables. The independent variable was time (pre-solution (PRE), 1 h, 3 h, 6 h, 12 h, and 24 h post-solution administration) and the dependent variables were stretch reflex, response to pinch, response to mechanical flick, and response to light touch. Statistical significance was set a priori at $P < 0.05$. Dunn's multiple comparison test was performed for any significant differences.

With respect to the control animals, preliminary work demonstrated no significant difference in either behavioral assessments or EMG activity between animals that received a laminectomy-only surgery versus those who receive no surgery. Thus, for statistical purposes these two groups were combined to establish one control group. With the exception of one animal all animals underwent behavioral assessment following administration of both saline (C-S, $n=9$) and gabapentin (C-GBP, $n=10$). With respect to the SCI animals, 17 SCI spastic animals were behaviorally assessed following administration of saline (spinal cord-injured animal receiving saline, SCI-S) and GBP (spinal cord injured animal receiving gabapentin, SCI-GBP).

EMG assessment. To determine the effect of saline or GBP on EMG activity in control animals (C-S ($n=9$) and C-GBP ($n=7$)) a repeated measures ANOVA with one within factor (time) and one between factor (group) was performed. The time factor had six levels (PRE 1 h, 3 h, 6 h, 12 h, and 24 h post-solution administration), and the between-factors were the pharmacologic agent (saline or GBP). The dependent variable was the 6 s of average EMG RMS amplitude following noxious pinch stimulus represented as % MA.

To determine the effect of saline or GBP on SCI-induced spasticity a repeated measures ANOVA with one within factor (time) and one between factor (group) was performed. The time factor had the same six levels as above, the between group factor had three levels (SCI-GBP ($n=11$), SCI-S ($n=8$), and C-S ($n=9$)). Statistical significance was set a priori at $P < 0.05$ for both procedures. Bonferroni post hoc analysis was performed for any significant difference.

With respect to the control animals, seven were assessed for changes in EMG activity following administration of GBP, while all nine C-S animals that were behaviorally assessed were also assessed for changes in EMG activity following administration of saline and GBP. With respect to the SCI animals, of the 17 animals behaviorally assessed post-drug administration, 8 were assessed by EMG following administration of both saline and GBP, while an additional 3 animals were assessed following administration of GBP only.

RESULTS

Effects of GBP on the behavior and EMG activity in control animals

Behavioral response. The results of the Kruskal-Wallis tests revealed no significant difference between the C-S and C-GBP groups with respect to response to stretch reflex, pinch, mechanical flick, or light touch for any of the six time conditions. Prior to saline or GBP administration

the baseline level of responsiveness for each of the behaviors was between 0 and <0.5 (out of 5.0) (Fig. 2). Following administration of saline a small increase in responsiveness, between 0.14 and 0.42 (out of 5.0), was seen at 3, 6 and 12 h post-injection with respect to stretch and pinch, and at 3 and 6 h with respect to mechanical flick. With respect to light touch, a minimal increase in responsiveness was seen at 3 h post-injection (Fig. 2). Thus, control animals displayed very little responsiveness to stretch, pinch, mechanical flick, and light touch before and after administration of saline. Following administration of GBP there was no change in baseline behavioral activity at 1, 3, and 6 h post-injection (Fig. 2). At 12 and 24 h post-injection a small, but not statistically significant increase in behavioral responsiveness (≤ 0.25 of 5.0) was seen with respect to stretch, pinch, mechanical flick, and light touch (Fig. 2). Thus, administration of GBP had no significant effect on the responsiveness to stretch, pinch, mechanical flick, or light touch in control animals. GBP administered at 50 mg/kg did not produce any overt indications of locomotor ataxia or lethargy in the control animals.

Mean EMG activation. No significant differences were observed in % MA between PRE and any of the five post-administration time conditions for either the C-S or C-GBP groups. In addition, no significant differences were observed in the % MA when the C-S group was compared with the C-GBP group at each of the six time conditions. Therefore, in further analysis only the C-S group was used to represent the control condition in comparison to SCI groups.

Effects of GBP on behavior and EMG activity in SCI spastic animals

Behavioral response. Prior to administration of saline the SCI spastic animals displayed a baseline level of responsiveness for stretch, pinch, and mechanical flick of 4.8, 4.7, 3.5 (out of 5.0), respectively, and light touch was 1.37 (out of 2.0). Each of these behavioral levels was significantly larger than that of control animals ($P < 0.001$). The Kruskal-Wallis tests revealed no significant difference between time conditions with respect to responsiveness to stretch reflex, pinch, mechanical flick or light touch following administration of saline (Fig. 3). Prior to administration of GBP the baseline level of responsiveness for stretch, pinch, mechanical flick, and light touch was 4.5, 4.7, 4.3 (out of 5.0), and 1.5 (out of 2.0), respectively. The results of the Kruskal-Wallis tests revealed a significant difference between time conditions following administration of GBP. Dunn's multiple comparison test revealed a significant difference between the PRE condition and the 1 h and 3 h ($P < 0.001$) post-administration conditions for each of the behaviors tested. This difference was represented as a significant decrease in responsiveness as measured by each of the behavioral assessments (Fig. 3). GBP administered at 50 mg/kg did not produce any overt indications of locomotor ataxia or lethargy in SCI animals.

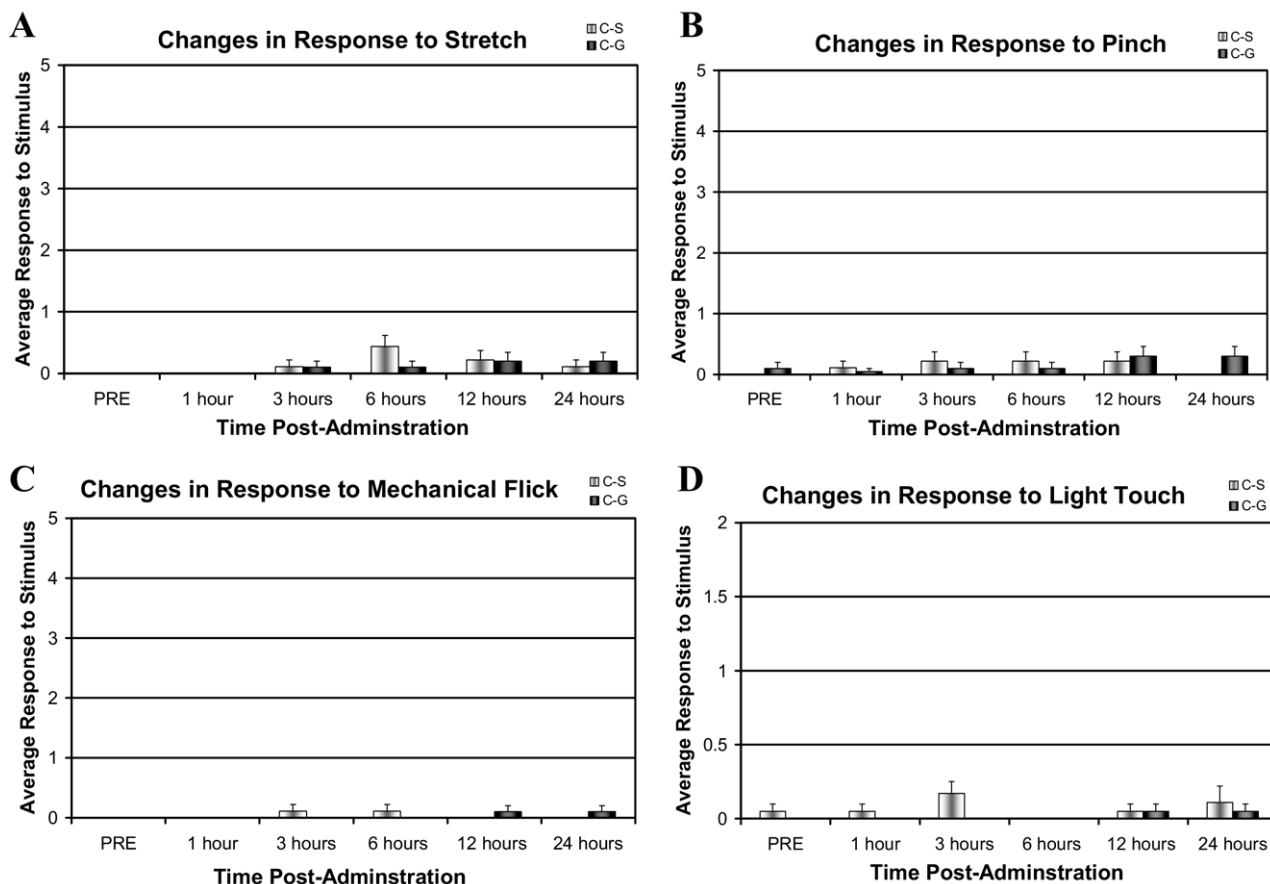


Fig. 2. The average behavioral response of the tail musculature to a quick stretch (Fig. 2A), pinch (Fig. 2B), mechanical flick (Fig. 2C), or light touch (Fig. 2D) in control animals following the administration of saline (C-S) or GBP (C-G; 50 mg/kg). Response to stretch, pinch, and mechanical flick was scored on a 5-point scale in which 0=no spasticity and 5=severe spasticity. Light touch was score on a 2-point scale in which 0=no response to light touch and 2=significant flexion away from the stimulus. Notice that control animals displayed minimal or no response to any of the stimuli pre or post-administration of either saline or GBP. Data were analyzed using Kruskal-Wallis nonparametric tests followed by post hoc analysis with Dunn's multiple comparison test.

Mean EMG activation. The results of the repeated measures ANOVA revealed a significant group \times time interaction ($F=4.52$, 10 *df*, $P<0.001$). Bonferroni post hoc analysis revealed the SCI-S group demonstrated significantly higher % MA values than the C-S group at the PRE, 1 h, 3 h, 6 h, and 12 h post-solution conditions (Fig. 4). The SCI-GBP group exhibited significantly higher % MA values than the C-S group at the PRE, 6 h, 12 h, and 24 h post-solution time conditions, but not at 1 and 3 h. Finally, the SCI-GBP group demonstrated significantly lower % MA values than the SCI-S group at the 1 h and 3 h time conditions but not at later time points ($P<0.001$) (Fig. 4).

DISCUSSION

The present study was a blinded, placebo-controlled crossover study, which investigated the potential efficacy of GBP as a treatment for SCI-related spasticity in the tail musculature. However, in order to more clearly understand the effects of GBP on the behavior and EMG activity in SCI spastic animals, the effects of GBP on the behavior and EMG activity in control (non-SCI) animals was first exam-

ined in order to establish the baseline "normal" tail activity before and after administration of GBP.

Effects of GBP in control animals

Overall, control animals (no surgery and laminectomy-only) displayed minimal behavioral response to stretch and cutaneous (noxious and non-noxious) stimulation. These results correspond to those of several previous studies, which reported minimal change in behavioral and/or EMG activity in the rat tail muscles of non-injured animals in response to stimulation (Bennett et al., 1999; Kitzman, 2005, 2006). The administration of saline had no significant effect on tail behavioral or EMG activity; while the administration of GBP decreased both behavioral and EMG activity. However, since the baseline level of activity was low to begin with, the effects of GBP on this baseline activity were not significant.

Effects of GBP in SCI spastic animals

Results from the present study demonstrated both baseline (pre-drug) and post-saline levels of behavioral and

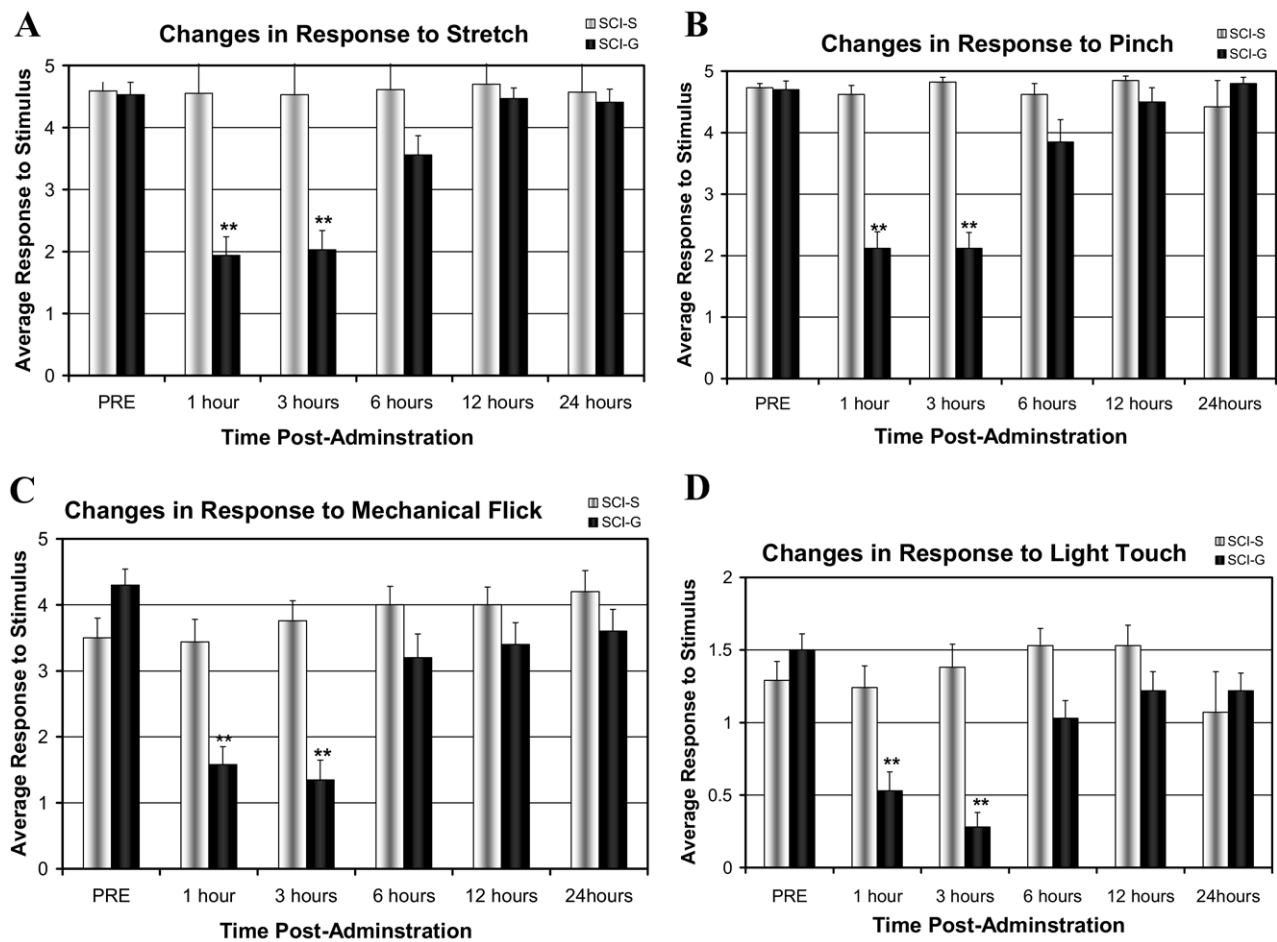


Fig. 3. The average behavioral response of the tail musculature to a quick stretch (Fig. 3A), pinch (Fig. 3B), mechanical flick (Fig. 3C), or light touch (Fig. 3D) in SCI animals following the administration of saline (SCI-S) or GBP (SCI-G; 50 mg/kg). Notice that SCI animals displayed a significantly larger spastic response to each of the stimuli, both pre- and post-administration of saline, compared with control animals. Prior to administration of GBP, SCI animals displayed a significantly larger spastic response to each of the stimuli compared with control animals. At 1 and 3 h post-administration of GBP there was a significant decrease in the responsiveness of the tail musculature to each of the stimuli. Data were analyzed using Kruskal-Wallis nonparametric tests followed by post hoc analysis with Dunn's multiple comparison test (** $P < 0.01$).

EMG activity in the tail musculature was significantly increased in SCI animals when compared with control animals. This increase in tail musculature activity, post-transection, has been previously reported (Bennett et al., 1999, 2004; Kitzman, 2005, 2006). Following administration of GBP (50 mg/kg), the observed increased responsiveness to a quick stretch of the tail, noxious (pinch) and non-noxious (light touch) cutaneous stimulation were all significantly decreased. Thus, it appears GBP is capable of suppressing multiple aspects of spasticity. In addition, data from the present study suggest the therapeutic window for suppressing spasticity in the tail musculature is at least 3 h post-administration; gradually decreasing in effectiveness over the subsequent 6 h. This therapeutic time frame is similar to that previously shown in clinical studies (Goa and Sorkin, 1993; McLean, 1994).

Previous studies have demonstrated GBP administration can lead to a minor to moderate incidence of side-effects (e.g. somnolence, dizziness, ataxia) (Francisco et al., 2001; McLean, 1995; McLean and Gidal, 2003; McLean

et al., 1999). However, these studies also demonstrated GBP to be well tolerated and reported a low incidence of serious side-effects. The dosage of GBP used in the present study (50 mg/kg) was based upon the well-characterized effects of this dosage in rat models of neuropathic pain and epilepsy (De Vry et al., 2004; Hofmann et al., 2003). At this dosage there were no overt signs of gait ataxia or sedation. While the dosage used in the present study was higher than the typical clinically administered dosage, it was within the therapeutic range previously reported for the treatment of epilepsy in the pediatric population (Gatti et al., 2003; McLean and Gidal, 2003). In addition, multiple animal studies examining neuropathic pain have used concentrations of GBP that were significantly higher than the dosage used in the present study with few reported side-effects (Coderre et al., 2005, 2007; Gaida et al., 2005; LaBuda and Little, 2005; Pedersen and Blackburn-Munro, 2006).

Both semi-quantitative and quantitative measures were used in the present study to demonstrate GBP significantly

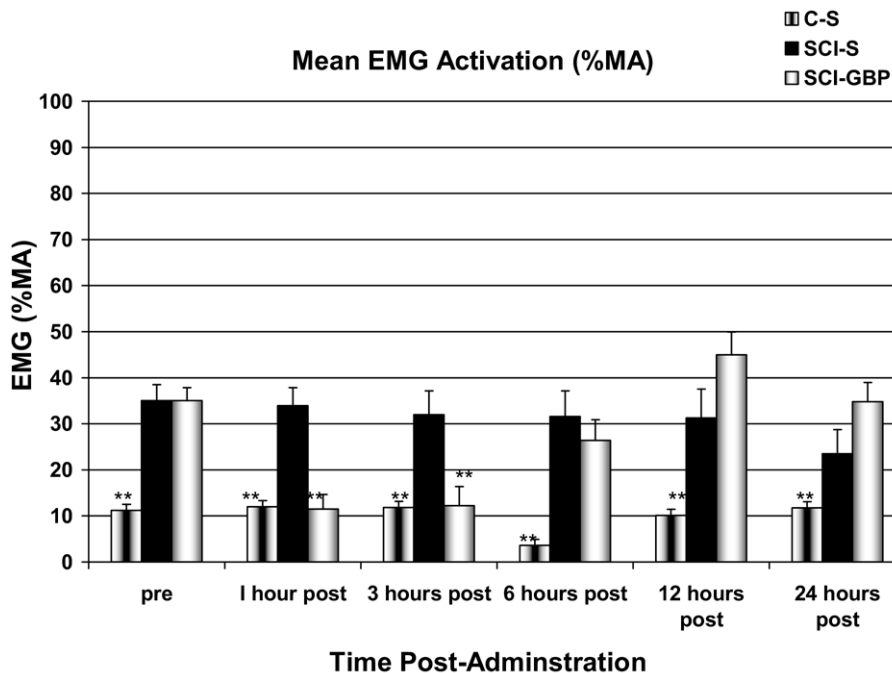


Fig. 4. Percent of the mean EMG response (% MA) of the tail musculature to pinch stimulation in control animals following administration of saline (C-S) and SCI animals following administration of either saline (SCI-S) or GBP (SCI-GBP; 50 mg/kg). Notice control animals displayed a significantly lower % MA compared with SCI animals. SCI animals demonstrated no significant changes in % MA following administration of saline. However, SCI animals demonstrated a significant decrease in % MA at 1 and 3 h post-administration of GBP (** $P < 0.001$). Data were analyzed using one-way ANOVA followed by a Bonferroni post hoc analysis.

suppresses both behavioral and electrophysiological manifestations of spasticity. However, the present study examined only one dosage of GBP. Additional dose response studies will be required in order to establish the maximal therapeutic dosage and duration for this pharmacologic agent in the management of SCI-induced spasticity in the S_2 transection model. These studies will provide a greater understanding of the efficacy of suppressing glutamate release as an adjunct treatment for the management of spastic behavior in a less complex model of SCI-induced spasticity, which will provide a baseline level of understanding that will be crucial when examining the efficacy of this treatment in managing spastic behavior in more complex spinal systems (e.g. circuitry involved with locomotion and upper extremity function).

The present study was interested in examining changes in EMG activity over time due to its potential clinical relevance in restricting mobility. A momentary (ms) maximal event of electrical activity is not necessarily adequate to cause a clinical restriction of motion. Therefore, it was of interest to examine the electrophysiological response to a stimulus for a longer window of time. In the present study it was determined that following the application of a pinch stimulus, EMG activity in the tail musculature of SCI spastic animals remained significantly elevated for approximately 6 s post-stimulus. The same stimulus applied to the tail of control (uninjured) animals produced and increased EMG activity lasting approximately 1 s. The significant increase in the duration of EMG activity in the SCI animals would be considered a clinically relevant time period that could result

in significant functional restriction. Administration of GBP effectively reduced the level of EMG activity to control levels and maintain this decreased level of activity for 3–6 h post-injection. Thus, with respect to the tail musculature, GBP is effective in managing the SCI-induced increase in muscle activity. However, as stated previously, the responsiveness of the tail muscles to GBP may not represent the same changes in muscle activity that would be seen with the limb musculature. This will be the focus of future studies.

Relationship to GBP effects in clinical trials

Several clinical studies have examined the usefulness of GBP in the management of spasticity arising from either multiple sclerosis or SCI origins (Cutter et al., 2000; Grunthal et al., 1997; Mueller et al., 1997; Priebe et al., 1997). These studies demonstrated only a modest improvement in spastic behavior following administration of GBP. However, these clinical studies were not designed to evaluate GBP as a single treatment for spasticity as demonstrated by over 80% of the subjects taking at least one other anti-spasticity medication. Additionally, the dosages were not standardized to body weight. Thus, each of the subjects could potentially have received a significantly different overall dosage of GBP, which would make interpretation of results problematic. Thus, the data presented in previous clinical studies could only be taken as evidence supporting the potential use of GBP as adjunct therapy in the management of spasticity.

CONCLUSION

In the present study it was demonstrated that administration of GBP significantly decreased both the behavioral and electrophysiological manifestations of spasticity in the tail musculature.

Thus, suppression of glutamate release may potentially be an effective treatment for SCI-induced spasticity. However, further studies are required to fully understand the interaction between the glutamatergic system and the development of spasticity in order to more clearly evaluate the potential efficacy of suppressing this neurotransmitter system (with pharmacologic agents such as GBP) for the management of spasticity.

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