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Combinations of intrathecal gamma-amino-butyrate receptor agonists and N-methyl-D-aspartate receptor antagonists in rats with neuropathic spinal cord injury pain

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Abstract

Underlying below-level cutaneous hypersensitivity observed following spinal cord injury (SCI) is a concurrent loss of inhibition with an increase in excitation in the spinal dorsal horn. Thus, a dual pharmacological approach, increasing spinal γ -aminobutyrate (GABA) inhibition and decreasing N-methyl-D-aspartate (NMDA) receptor-mediated excitation, could be more beneficial than either approach alone. The current study evaluated the antinociceptive effects of lumbar intrathecal (i.t.) administration of GABA receptor agonists and NMDA receptor antagonists alone and in combination in rats with neuropathic SCI pain. Rats developed markedly decreased hind paw withdrawal thresholds following an acute thoracic spinal cord compression, indicative of below-level hypersensitivity. Separately, i.t. GABA_A receptor agonist muscimol and GABA_B receptor agonist baclofen demonstrated dose-dependent antinociception, whereas i.t. NMDA receptor antagonist ketamine and the endogenous peptide [Ser¹]histogranin, a putative NMDA receptor antagonist, demonstrated no efficacy. The combination of baclofen and ketamine resulted in a supra-additive (synergistic) antinociception whereas the combinations with muscimol were merely additive. Intrathecal pretreatment with the GABA_B receptor antagonist CGP 35348 prevented the antinociceptive effect of the baclofen and ketamine combination. The data indicate that blocking spinal NMDA receptors alone is not sufficient to ameliorate SCI hypersensitivity, whereas a combined approach, simultaneous activation of spinal GABA_B receptors and NMDA receptors blockade with ketamine, leads to significant antinociception. By engaging diverse pain modulating systems at the spinal level, combination drug treatment may be a useful approach in treating neuropathic SCI pain.

Keywords

allodynia; baclofen; combination drug therapy; histogranin; ketamine; muscimol

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1. Introduction

Central sensitization is abnormal physiological activity of spinal dorsal horn sensory neurons, which include elevated and prolonged responsiveness following peripheral cutaneous stimulation (Millan, 1999). Sensitized dorsal horn neurons are believed to underlie neuropathic spinal cord injury (SCI) pain, and like peripheral neuropathic pain, symptoms include spontaneous pain and cutaneous hypersensitivity (Finnerup et al., 2007; Rowbotham and Fields, 1996). Interestingly, SCI patients report significant neuropathic pain below the level of the injury which is difficult to treat and elucidate in terms of mechanism (Cruz-Almeida et al., 2005).

One significant change following SCI in dorsal horn neurochemistry in rats is the loss of γ -aminobutyrate (GABA)-mediated inhibition (Gwak and Hulsebosch, 2011a). The loss of GABAergic inhibition following SCI could be due to a diminished capacity of spinal interneurons to synthesize GABA or an outright loss of these neurons. Simultaneously, there is increased glutamate receptor-mediated excitation, particularly by the *N*-methyl-D-aspartate (NMDA) glutamate receptor subtype, of dorsal horn sensory neurons (Faden et al., 1988; Gwak and Hulsebosch, 2011b). The concurrent neurochemical imbalance of inhibition and excitation is presumed to play a prominent role in the mechanism underlying spontaneous and evoked pains following SCI.

Although systemic treatment with NMDA receptor antagonists and GABA receptor agonists have demonstrated efficacy in experimental neuropathic SCI pain, adverse side-effects induce muscle weakness, sedation and psychomimetic effects (Gracies et al., 1997; Hama and Sagen, 2007; Hao et al., 1991; Max et al., 1995; Xu et al., 1993). As an alternative, drugs could be intrathecally (i.t.) injected, thereby reducing the potential for supraspinally mediated side-effects (Bennett et al., 2000b). Furthermore, drugs could be dosed in combination to engage multiple pain-related mechanisms (Mao et al., 2011; Raffa et al., 2003). The simultaneous targeting of complementary mechanisms could result in synergism, wherein the efficacy of the combination is significantly greater than those of the constituents alone. For example, i.t. morphine and clonidine combined reportedly gave better analgesia in SCI pain patients than either drug alone (Siddall et al., 2000).

Another potential long-term solution is engineered cell lines that express endogenous analgesic substances (Hentall and Sagen, 2000; Jeon, 2011). For example, adrenal medullary chromaffin cells release opioid peptides and catecholamines. When implanted into the lumbar subarachnoid space, these cells give long-lasting opioid receptor- and α -adrenoceptor-mediated antinociception (Hentall and Sagen, 2000). The adrenal medulla also synthesizes [Ser¹]histogranin, a 16-amino acid peptide with a number of pharmacological properties, including possible non-competitive antagonism of the NMDA receptor (Lemaire et al., 1993; Shukla et al., 1995). [Ser¹]histogranin has demonstrated antinociceptive activity in pre-clinical pain models, presumably through the blockade of the NMDA receptor (Hama and Sagen, 2002; Hama et al., 1999; Hentall et al., 2007; Siegan et al., 1997). [Ser¹]histogranin alone has yet to be demonstrated in rats with neuropathic SCI pain and, furthermore, combining [Ser¹]histogranin with other analgesic substances, with the potential for synergy, has yet to be tested.

The current study evaluated the antinociceptive potential of i.t. mixtures of GABA receptor agonists and NMDA receptor antagonists and compared their effects to that of separate i.t. treatments in a rat model of neuropathic pain following an acute spinal compression.

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighed 100–150 g upon arrival to the vivarium. Rats were housed two per cage in a climate-controlled room, on a 12 hr light-dark cycle, with free access to food and water and were allowed at least five days to acclimate to the facility. All experimental procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences) and were approved by the University of Miami Animal Care and Use Committee.

2.2. Animal Surgery

Aseptic technique, including the use of sterile surgical instruments, sterile gloves and appropriate personal protective equipment, was used with all surgical procedures. Prior to surgery, rat hairs were shaved and the surgical area swabbed with chlorhexidine. Rats were rendered unconscious and maintained on isoflurane in O₂ during the surgical procedures.

2.2.1. Spinal Cord Injury—The procedure to induce a spinal cord compression injury has been previously described (Hama and Sagen, 2007). The effect of compression injury on rat spinal tissue, in contrast to, for example, a spinal hemisection, closely resembles that of clinical SCI (Bruce et al., 2002; Bunge et al., 1993). Acute spinal compression in rats also induces a long-lasting below-level hypersensitivity to cutaneous stimuli similar to what is observed in clinical SCI (Finnerup et al., 2003).

Briefly, a laminectomy was performed to expose the mid-thoracic spinal segments T₆–T₇ and the dura was left intact. A microvascular clip (Harvard Apparatus; Holliston, MA) was placed vertically over the exposed thoracic spinal cord and left in place for 60 sec. Care was taken not to cut the dura or disturb nearby spinal nerve roots. Following removal of the clip, the overlying muscles were sutured closed with Vicryl and the skin closed with wound clips. Bladder function spontaneously returned in these rats 1 to 2 days after surgery.

2.2.2. Intrathecal catheterization—Three weeks after SCI surgery, each SCI rat was given an indwelling intrathecal (i.t.) catheter (32-g; ReCathCo, Allison Park, PA) using a previously described procedure (Yaksh and Rudy, 1976). The dorsal neck musculature was exposed and the atlantooccipital membrane was exposed. A catheter was threaded down the i.t. space. The length of the internalized catheter was about 8.5 cm and terminated at the level of lumbar enlargement, caudal to the SCI. The externalized end of the catheter was sutured to the musculature. The skin was sealed shut with veterinarian grade cyanoacrylate. Following a saline flush of the catheter, the externalized end was melted shut.

Following cannulation surgery, rats were individually housed and had free access to food and water. Rats were allowed seven days to recover from surgery prior to use in behavioral studies; rats were tested four weeks following SCI surgery.

At the end of the experiment, the placement of the catheter tip relative to lumbar spinal cord was confirmed by i.t. injection of 5 µl of 5% lidocaine. A rapid bilateral hind limb paralysis indicated proper placement of the catheter. Rats were then euthanized with an overdose of CO₂.

2.3. Experimental Procedure

2.3.1. Hind paw mechanical stimulation—To measure hind paw sensitivity to innocuous mechanical stimulation, the withdrawal thresholds (in grams) were measured with a set of eight specific von Frey filaments using the up-down method (Chaplan et al., 1994).

Four weeks after spinal compression, prior to drug injection, withdrawal thresholds were measured. Rats were placed in Plexiglas containers with a wire mesh floor and allowed 20 min to acclimate. The pattern of responses to the filaments determined the withdrawal threshold. Rats that did not respond to the highest force filament were assigned a threshold of 15 g. Before SCI surgery, rats did not respond to the highest force filament. To be included in the study, the hind paw withdrawal threshold of SCI rats needed to be 4 g or less.

2.3.2. Intrathecal drug testing—Following baseline threshold measurement, SCI rats were injected with either drug, combination of drugs or saline (vehicle) in a total volume of 5 μ l, followed by a 5 μ l vehicle flush, and tested once every 30 min for up to 120 min post-injection.

For pre-treatment with the GABA_B receptor antagonist (3-Aminopropyl)(diethoxymethyl) phosphinic acid (CGP 35348), baseline thresholds were recorded, then CGP 35348 was injected. Thirty min following antagonist injection, the drug combination was injected and rats were tested 30 min thereafter. (Rats were tested 60 min after i.t. antagonist injection.) In the antagonist pretreatment study, there were four treatment groups (pre-treatment/post-treatment): Vehicle/Vehicle; Vehicle/Combination; CGP 35348/Vehicle; CGP 35348/Combination.

In a separate study, the antagonistic effect of i.t. 30 μ g CGP 35348 on i.t. 1 μ g baclofen was tested in a similar manner, using the same time course, to confirm that the antinociceptive effect of baclofen was mediated by spinal GABA_B receptors and to confirm that the dose of antagonist was sufficient. (A similar dose of CGP 35348 was used in other studies to antagonize baclofen (Dirig and Yaksh, 1995; Hammond and Washington, 1993).) There were four treatment groups: Vehicle/Vehicle; Vehicle/Baclofen; CGP 35348/Vehicle; CGP 35348/Baclofen.

2.4. Drugs

All reagents and drugs, except where noted, were obtained from Sigma-Aldrich, Co. (St. Louis, MO) and all drugs were dissolved and diluted with saline. The doses used were: GABA_A receptor agonist muscimol HBr (0.1, 0.3, 1, 3 μ g), GABA_B receptor agonist ($\pm$)baclofen (0.1, 0.3, 1 μ g), GABA_B receptor antagonist CGP 35348 (30 μ g), NMDA receptor antagonist ketamine HCl (30, 100 μ g (Chaplan et al., 1997)); Fort Dodge Animal Health, IA), [Ser^1]histogranin (1, 3, 10 μ g; Bachem, Torrance, CA). The dose range of [Ser^1]histogranin has been shown to produce antinociceptive effects in peripheral neuropathic and inflammatory pain models (Hama and Sagen, 2002; Siegan et al., 1997). As a comparator to the NMDA receptor antagonists used in the current study, the non-competitive NMDA receptor antagonist (5*S*,10*R*)-(+)-5-Methyl-10,11-dihydro-5*H*-dibenzo[*a,d*]cyclohepten-5,10-imine maleate (dizocilpine (MK-801) 3, 10, 30 μ g) was i.t. injected in a separate group of SCI rats.

The drugs were combined following determination of the 50% antinociceptive doses (A_{50}) of muscimol and baclofen from the dose-response curves. Neither i.t. ketamine nor [Ser^1]histogranin demonstrated significant efficacy (see Results 3.2). Thus, the highest tested doses of ketamine (100 μ g) and [Ser^1]histogranin (10 μ g) were combined with the A_{50} doses of muscimol and baclofen. Muscimol was combined with ketamine (muscimol +ketamine): 1.8+100 μ g, 0.6+30 μ g, 0.3+10 μ g. Muscimol was combined with [Ser^1]histogranin (muscimol+[Ser^1]histogranin): 1.8+10 μ g, 0.6+3 μ g, 0.3+1 μ g. Baclofen was combined with ketamine (baclofen+ketamine): 0.3+100 μ g, 0.1+30 μ g, 0.03+10 μ g. Baclofen was combined with [Ser^1]histogranin (baclofen+[Ser^1]histogranin): 0.3+10 μ g, 0.1+3 μ g, 0.03+1 μ g.

2.5. Statistical Analysis

The drug effect on withdrawal threshold was converted to a percent maximum possible effect (percent MPE):

$$\text{Percent MPE} = ((\text{Postdrug threshold} - \text{Predrug Baseline threshold}) \div (15 \text{ g} - \text{Predrug Baseline threshold})) * 100.$$

Where applicable, the A_{50} (50% antinociceptive dose) and 95% confidence limits (C.L.) of the drugs were calculated from the linear portions of the log dose-response curves using a Web-based program, based on a previously published program (Tallarida and Murray, 1981).

In a typical case of determining synergism of a two-drug combination, both drugs are active and the A_{50} of each drug are plotted on an Cartesian plot to determine the line of additivity. The line connecting the A_{50} values is the theoretical additive line. If the A_{50} calculated from the experimentally determined dose-response curves (the isobol) falls below the line of additivity, the combination is supra-additive (synergistic) whereas if the A_{50} value falls on the line, the combination is merely additive (Tallarida, 2007; Tallarida et al., 1989). The experimentally determined A_{50} value may fall above the line of additivity, which indicates that the combination is sub-additive—one constituent is possibly antagonistic to the other. If experimental $A_{50} < \text{additive } A_{50}$ (t -test, $P < 0.05$), then the combination is considered synergistic.

In the current two-drug combination study, it was possible to generate a dose-response curve for only one of the drugs of the combination. Thus, a modified isobolographic analysis was needed to determine synergistic interaction. In this case, to calculate the theoretical additive A_{50} :

$$\text{additive } A_{50} = \text{active drug } A_{50} / P,$$

where P is the proportion of the combination that is the active drug. A significant (t -test $P < 0.05$) leftward shift of the dose-response curve of the combination compared to the dose-response curve of the active drug alone indicates synergy (Hama and Sagen, 2010; Porreca et al., 1990; Raffa et al., 2003; Tallarida, 2007). The theoretical and experimental A_{50} doses in Table 2 are the total amount of constituents in the mixture.

Comparisons over time between drug and vehicle-treated groups were performed using a repeated-measure two-way ANOVA with Student-Newman-Keuls test for *post hoc* comparisons (SigmaStat 3.1). Statistical analysis of antagonist pretreatment on the antinociceptive effect of the combination was performed using a two-way ANOVA. Statistical significance was taken at $P < 0.05$. Data are expressed as mean $\pm$ S.E.M.

3. Results

Prior to SCI surgery, the mean withdrawal threshold of all rats was 15 g (open squares at Pre-Injury (PI), Fig. 1A, 1B).

Four weeks after spinal compression, prior to i.t. drug injection, the average withdrawal threshold ($\pm$ S.E.M.) was 2.4 ± 0.2 g, a marked decrease from pre-injury thresholds of 15.0 (± 0.0) g ($P < 0.05$, pre-injury vs. post-compression injury).

3.1. Effects of intrathecal injection of GABA receptor agonists

The A_{50} doses of the drugs i.t. injected alone are summarized in Table 1.

The effects of GABA receptor agonists on withdrawal thresholds over time in SCI rats following i.t. injection are shown in Fig. 1. The peak antinociceptive effect of the highest tested dose of muscimol (3 µg) was observed at 30 min post-i.t. injection and a significant antinociception lasted at least 120 min post-injection (Fig. 1A). There was a dose-dependent increase in withdrawal thresholds following i.t. injection of muscimol ($P < 0.05$). The percent MPE ($\pm$ S.E.M.) of the highest tested dose of muscimol was $58.5 \pm 12.8\%$.

A peak antinociceptive effect of 1 µg baclofen was observed 30 min post-i.t. injection and the antinociception lasted at least 120 min post-injection (Fig. 1B). The antinociceptive effect of i.t. baclofen was dose-dependent ($P < 0.05$). By visual non-quantitative observation, baclofen at 1 µg did not appear to significantly affect hind limb motor tone (Dirig and Yaksh, 1995). At 30 min post-injection, the percent MPE was $85.9 \pm 9.7\%$.

Thirty min following injection, the A_{50} (95% C.L.) for muscimol is 1.8 (1.4) µg and that of baclofen is 0.3 (0.1) µg; baclofen is about six-fold more potent than muscimol (per weight). Fig. 3A and Fig. 3B show the dose-response curves for muscimol and baclofen alone (dashed lines), respectively. Injection of vehicle did not significantly alter withdrawal thresholds.

3.2. Effects of intrathecal injection of NMDA receptor antagonists

The effects of NMDA receptor antagonists and [Ser¹]histogranin on withdrawal thresholds over time in SCI rats following i.t. injection are shown in Fig. 2. In contrast to the efficacy obtained with i.t. GABA receptor agonists, there was a lack of significant dose-dependent efficacy with the NMDA receptor antagonists and [Ser¹]histogranin.

Intrathecal injection of 100 µg ketamine did not significantly increase withdrawal thresholds in SCI rats in the doses tested ($P > 0.05$; Fig. 2A). The percent MPE of ketamine 30 min post-i.t. injection was $4.1 \pm 2.7\%$. Although increased withdrawal thresholds were observed with 10 µg [Ser¹]histogranin, this was not statistically significant ($P > 0.05$; Fig. 2A). The percent MPE of [Ser¹]histogranin 30 min post-i.t. injection was $31.9 \pm 14.4\%$. Intrathecal injection of vehicle did not significantly alter withdrawal thresholds.

A non-dose-dependent antinociceptive effect of MK-801 was observed following i.t. injection (Fig. 2B). Intrathecal injection of 30 µg MK-801 resulted in a percent MPE of $52 \pm 14\%$. Whereas i.t. injection of 30 µg MK-801 led to psychomotor side-effects, side-effects were not observed following i.t. injection of either ketamine or [Ser¹]histogranin (Chaplan et al., 1997; Hama and Sagen, 2002; Hama et al., 2003).

3.3. Combinations of intrathecal GABA receptor agonists and NMDA receptor antagonists

The combination of muscimol with [Ser¹]histogranin was merely additive (Fig. 3A, Table 2; $P > 0.05$ vs. theoretical A_{50}). At the highest tested dose, the percent MPE of muscimol+[Ser¹]histogranin (1.8+10 µg) was $37.5 \pm 14\%$. The combination of muscimol and ketamine was not synergistic, but the observed A_{50} of this combination was slightly higher than the expected A_{50} of muscimol and ketamine. At the highest tested dose, the percent MPE of muscimol+ketamine (1.8+100 µg) was $14.0 \pm 8.5\%$. Thus, the combination of muscimol +ketamine was also merely additive ($P > 0.05$ vs. theoretical A_{50}).

By contrast, the combination of baclofen with ketamine (baclofen+ketamine: 0.03+10 µg, 0.1+30 µg, 0.3+100 µg) was synergistic (Fig. 3B, Table 2; $P < 0.05$ vs. theoretical A_{50}). The combination of baclofen with [Ser¹]histogranin was merely additive ($P > 0.05$ vs. theoretical A_{50}). At the highest tested dose, the percent MPE of baclofen+[Ser¹]histogranin (0.3+10 µg) was $50.2 \pm 14.6\%$.

3.4. Block of the antinociceptive effect of baclofen alone and baclofen+ketamine combination with CGP 35348

Intrathecal pretreatment with 30 μ g of CGP 35348 was sufficient to block the antinociceptive effect of 1 μ g baclofen in SCI rats (Fig. 4A; $P < 0.05$ CGP 35348/Baclofen vs. Vehicle/Baclofen). The mean withdrawal threshold of the CGP 35348/baclofen treatment group was similar to that of the Vehicle/vehicle treatment group ($P > 0.05$). Intrathecal pretreatment with vehicle did not affect the antinociceptive effect of baclofen ($P < 0.05$ Vehicle/Baclofen vs. Vehicle/Vehicle). Also, i.t. pretreatment with CGP 35348 and i.t. posttreatment with vehicle did not increase withdrawal thresholds—CGP 35348 was not antinociceptive in SCI rats. The data indicated that the antinociceptive effect of i.t. baclofen was mediated by dorsal horn GABA_B receptors.

Intrathecal pretreatment with CGP 35348 completely blocked the antinociceptive effect of the baclofen+ketamine (0.3+100 μ g) combination (Fig. 4B; interaction between CGP 35348 $\times$ combination, $P < 0.05$). These data indicated that the antinociceptive effect of the combination was mediated in part through spinal dorsal horn GABA_B receptors.

4. Discussion

The current study evaluated the possibility of simultaneous inhibition through GABA receptor-mediated activation and blocking NMDA receptor-mediated excitation at the level of spinal dorsal horn as a novel therapeutic strategy to ameliorate neuropathic SCI pain. While i.t. muscimol and baclofen separately demonstrated significant, dose-dependent antinociceptive effects in SCI rats, ketamine and [Ser¹]histogranin showed no activity. Interestingly, only the combination of baclofen with ketamine led to antinociceptive synergy, which was mediated through the spinal GABA_B receptor since i.t. pretreatment with CGP 35348 completely prevented the onset of antinociception. The results suggest that a combination of spinal NMDA receptor block and activation of GABA_B, rather than GABA_A receptors, with currently available drugs, will be most advantageous for neuropathic SCI pain.

Similarities in dorsal horn neurochemistry and physiology have been noted across the various neuropathic pain models (Gwak and Hulsebosch, 2011b; Millan, 1999). Following injury, increases in excitatory amino acids and other neuromodulatory substances lead to a state of facilitated and prolonged activation of dorsal horn neurons (Gwak and Hulsebosch, 2011b). At the same time, the inhibitory GABAergic system is significantly diminished following injury, either through cell death or significant attenuation of the synthesis of GABA by GABAergic interneurons (Drew et al., 2004; Gwak and Hulsebosch, 2011a; Zhang et al., 1994). The loss of GABAergic terminals originating from bulbospinal neurons within the dorsal horn also contributes to diminished GABAergic inhibition following spinal injury (McGowan and Hammond, 1993; Shapiro, 1997). Acute treatments that separately counteract these changes in neurochemistry lead to varying levels of antinociception in models of peripheral and central neuropathic pain (Bennett et al., 2000a; Chaplan et al., 1997; Dirig and Yaksh, 1995; Eaton et al., 1999; Hwang and Yaksh, 1997; Mao et al., 1993).

In the current study, an antinociceptive effect was not obtained with the clinically used NMDA receptor antagonist ketamine or with the endogenous peptide [Ser¹]histogranin. [Ser¹]histogranin has demonstrated antinociceptive effects in various animal models of cutaneous hypersensitivity via blockade of the NMDA receptor, but efficacy could also be mediated through other mechanisms (Hama et al., 1999; Hentall et al., 2007; Lemaire et al., 1995; Ruan and Lemaire, 2001). The lack of significant efficacy could be due to the absence in SCI rats of the substrate upon which [Ser¹]histogranin acts in other pain models.

Although i.t. NMDA receptor antagonists have been typically described as antinociceptive, on closer evaluation, the degree of antinociception is highly dependent on the compound, injury state and the tested sensory modality (Bennett et al., 2000a; Chaplan et al., 1997; Koizuka et al., 2005; Mao et al., 1993). Chaplan et al. (1997) found no significant antinociceptive effect of ketamine in nerve-injured rats and a MPE of less than 50% for MK-801. In spinally hemisectioned rats, Bennett et al. (2000a) demonstrated an antinociceptive effect of a competitive NMDA receptor antagonist on below-level mechanical but not heat hypersensitivity. Responses of lumbar wide dynamic neurons (WDR), below the level of hemisection, to cutaneous stimulation showed modest suppression (about 17%) following spinal application of a high dose (50 μ g) of MK-801 (Leem et al., 2010). (By contrast, block of 2-amino-3-(5-methyl-3-oxo-1,2-oxazol-4-yl) propanoate (AMPA) receptors led to a 50% suppression of WDR neuron responses.)

Greater efficacy has been observed with systemic administration of antagonist, which could be due to the recruitment of supraspinal and peripheral as well spinal NMDA receptors (however, see Chaplan et al., 1997). Likewise, the effect of i.t. MK-801 in the current study could be due to the recruitment of supraspinal and possibly peripheral NMDA receptors (Carlton, 2009). Although blocking supraspinal NMDA receptors could lead to antinociception, blockade these receptors would invariably lead to centrally mediated side-effects which may not be tolerable (Bennett et al., 2000b; Koizuka et al., 2005; Vranken et al., 2005).

The lack of efficacy of i.t. ketamine in the current study could be due to a similarly modest reduction in lumbar WDR neuron activity and the failure at the tested dose to recruit supraspinal or peripheral NMDA receptors. Recent studies in SCI rats have uncovered significant physiological changes in peripheral nerve and dorsal root ganglion neurons, similar to that observed following nerve injury, and these could be targeted by NMDA receptor antagonists (Bedi et al., 2010; Carlton et al., 2009). A peripherally mediated mechanism could in part explain the efficacy of intravenous ketamine in clinical neuropathic SCI pain (Kvarnstrom et al., 2004). Testing NMDA receptors expressed on peripheral nerves from SCI rats will be needed to confirm a role of these receptors in the maintenance of neuropathic SCI pain. If these receptors do play a significant role, then utilizing peripherally restricted antagonists could be a more patient-friendly treatment compared to centrally acting antagonists.

In contrast to the lack of efficacy of NMDA receptor antagonists, the current study demonstrated dose-dependent antinociceptive effects by activating spinal GABA_A and GABA_B receptors. Intrathecal injection of muscimol and baclofen induced marked antinociception, consistent with previous findings (Gwak and Hulsebosch, 2011a). Interestingly, the potency of i.t. baclofen in rats with a spinal compression was greater than that of muscimol, which was also the case in peripheral injury pain models (Dirig and Yaksh, 1995; Hwang and Yaksh, 1997). The potency and efficacy of baclofen could be due to an increase in spinal GABA_B binding sites following SCI (Kroin et al., 1993). The finding of i.t. baclofen efficacy in experimental neuropathic SCI pain has been confirmed in limited clinical neuropathic SCI pain studies (Herman et al., 1992; Saulino et al., 2009; Taira et al., 1995). There are currently no selective GABA_A receptor agonists for clinical pain relief. Modulation of the GABA_A receptor with the benzodiazepine midazolam appears to alleviate clinical peripheral neuropathic pain but positive effects in SCI pain have yet to be reported (Dureja et al., 2010). Benzodiazepines act by increasing the efficacy of GABA_A receptor-ion channel complex binding to endogenous GABA, thereby enhancing Cl⁻ current and hyperpolarization of neurons. There are, however, plausible alternative analgesic mechanisms (Ishizawa et al., 1997; Yaksh and Allen, 2004). Nonetheless, a previous study in SCI rats did not find an antinociceptive effect following systemic administration of

diazepam (Hama and Sagen, 2007). Clinical studies with a GABA_A receptor agonist would directly address the role of this receptor in SCI and other pain states (Knabl et al., 2008).

The current study evaluated the usefulness of targeting both spinal excitation and inhibition in neuropathic SCI pain compared to that of piecemeal treatment (Mao et al., 2011). Surprisingly, the current study found no further improvement of the antinociceptive effect of GABA_A receptor activation when combined with ketamine. The lack of synergism between muscimol and ketamine in the current study could be due to several possible mechanisms. There is a close, positive interaction between NMDA and GABA_A receptors, such that activation of one will lead to activation of the other (Chaudieu et al., 1994). Activation of the GABA_A receptor indirectly leads to increased glutamate receptor activity by increasing the synaptic concentration of glutamate (Erhardt and Engberg, 2000). Also, it is possible that GABA_A receptor distribution and function following SCI are significantly decreased (however see Drew et al., 2004)—a closer examination of GABA_A receptor function following a SCI will be needed to understand the lack of synergism between these receptors and NMDA receptors. Despite possessing a reasonable understanding of drug mechanism, as pointed out by Raffa, there are few combinations that actually demonstrate analgesic synergism (Raffa et al., 2003).

By contrast, robust synergism was obtained with spinal GABA_B receptor activation and NMDA receptor block. The synergistic antinociception of i.t. co-administered baclofen and ketamine was attenuated by CGP 35348 pretreatment, confirming mediation by spinal GABA_B receptors. It is speculated both receptor systems work in tandem to suppress increased intracellular Ca²⁺ and subsequent depolarization. Calcium ion entry through NMDA receptors is blocked by an NMDA receptor antagonist. GABA_B receptors inhibit voltage-gated Ca²⁺ channel function via inhibitory G-proteins (Menon-Johansson et al., 1993). The presynaptic and postsynaptic location of GABA_B receptors could also underlie the pharmacologic synergism with NMDA receptors, by inhibiting both presynaptic transmitter release and post-synaptic NMDA receptor-mediated excitation (Duguid and Smart, 2009; Towers et al., 2000).

An additive antinociception was observed, however, when baclofen was combined with [Ser¹]histogranin. This suggests that the action of [Ser¹]histogranin on the NMDA receptor significantly differs from that of ketamine despite previous characterization of [Ser¹]histogranin as a noncompetitive antagonist (Shukla et al., 1995). As mentioned earlier, [Ser¹]histogranin modulates other receptors, such as the NK₁ and dopamine receptors, which have currently undefined roles in the neuropathic SCI pain state.

The current data suggest that inactivation of spinal NMDA receptors alone is not sufficient for robust antinociception. To work around the absence of efficacy at a single target, NMDA receptor antagonists could be of greater utility as an adjuvant (Amr, 2010). While a synergistic effect was not obtained, an additive combination may still be clinically useful. For example, tolerance and withdrawal following long-term baclofen could be suppressed with an adjuvant such as ketamine or [Ser¹]histogranin (Ando et al., 2011; Coffey et al., 2002; Heetla et al., 2009).

The current study uncovered an acute, synergistic antinociception at the spinal level between the GABA_B receptor and the NMDA receptor. As an alternative to mechanical intrathecal infusion pumps, subarachnoid transplantation of GABAergic cells genetically modified to express natural neuromodulatory substances could be used for long-term pain relief (Eaton, 2006; Hentall and Sagen, 2000). Although [Ser¹]histogranin was not antinociceptive in the current study, perhaps other peptides that act upon other neurotransmitter systems could be evaluated for use in a cell-based treatment (Hama and Sagen, 2009; Olivera and Teichert,

2007). Thus, a number of combination treatment strategies have yet to be evaluated which may have utility in the treatment of SCI pain.

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References

- Amr YM. Multi-day low dose ketamine infusion as adjuvant to oral gabapentin in spinal cord injury related chronic pain: a prospective, randomized, double blind trial. *Pain Physician*. 2010; 13:245–249. [PubMed: 20495588]
- Ando Y, Hojo M, Kanaide M, Takada M, Sudo Y, Shiraishi S, Sumikawa K, Uezono Y. S(+)-Ketamine suppresses desensitization of gamma-aminobutyric acid type B receptor-mediated signaling by inhibition of the interaction of gamma-aminobutyric acid type B receptors with g protein-coupled receptor kinase 4 or 5. *Anesthesiology*. 2011; 114:401–411. [PubMed: 21245733]
- Bedi SS, Yang Q, Crook RJ, Du J, Wu Z, Fishman HM, Grill RJ, Carlton SM, Walters ET. Chronic spontaneous activity generated in the somata of primary nociceptors is associated with pain-related behavior after spinal cord injury. *J. Neuroscience*. 2010; 30:14870–14882.
- Bennett AD, Everhart AW, Hulsebosch CE. Intrathecal administration of an NMDA or a non-NMDA receptor antagonist reduces mechanical but not thermal allodynia in a rodent model of chronic central pain after spinal cord injury. *Brain Res*. 2000a; 859:72–82. [PubMed: 10720616]
- Bennett G, Serafini M, Burchiel K, Buchser E, Classen A, Deer T, Du Pen S, Ferrante FM, Hassenbusch SJ, Lou L, Maeyaert J, Penn R, Portenoy RK, Rauck R, Willis KD, Yaksh T. Evidence-based review of the literature on intrathecal delivery of pain medication. *J. Pain Symptom. Manage*. 2000b; 20:S12–S36. [PubMed: 10989255]
- Bruce JC, Oatway MA, Weaver LC. Chronic pain after clip-compression injury of the rat spinal cord. *Exp. Neurol*. 2002; 178:33–48. [PubMed: 12460606]
- Bunge RP, Puckett WR, Becerra JL, Marcillo A, Quencer RM. Observations on the pathology of human spinal cord injury. A review and classification of 22 new cases with details from a case of chronic cord compression with extensive focal demyelination. *Adv. Neurol*. 1993; 59:75–89. [PubMed: 8420126]
- Carlton SM. Peripheral NMDA receptors revisited - Hope floats. *Pain*. 2009; 146:1–2. [PubMed: 19767145]
- Carlton SM, Du J, Tan HY, Nesic O, Hargett GL, Bopp AC, Yamani A, Lin Q, Willis WD, Hulsebosch CE. Peripheral and central sensitization in remote spinal cord regions contribute to central neuropathic pain after spinal cord injury. *Pain*. 2009; 147:265–276. [PubMed: 19853381]
- Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. *J. Neurosci. Methods*. 1994; 53:55–63. [PubMed: 7990513]
- Chaplan SR, Malmberg AB, Yaksh TL. Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. *J. Pharmacol. Exp. Ther*. 1997; 280:829–838. [PubMed: 9023297]
- Chaudieu I, St-Pierre JA, Quirion R, Boksa P. GABAA receptor-mediated inhibition of N-methyl-D-aspartate-evoked [3H]dopamine release from mesencephalic cell cultures. *Eur. J. Pharmacol*. 1994; 264:361–369. [PubMed: 7698177]
- Coffey RJ, Edgar TS, Francisco GE, Graziani V, Meythaler JM, Ridgely PM, Sadiq SA, Turner MS. Abrupt withdrawal from intrathecal baclofen: recognition and management of a potentially life-threatening syndrome. *Arch. Phys. Med. Rehabil*. 2002; 83:735–741. [PubMed: 12048649]
- Cruz-Almeida Y, Martinez-Arizala A, Widerstrom-Noga EG. Chronicity of pain associated with spinal cord injury: A longitudinal analysis. *J. Rehabil. Res. Dev*. 2005; 42:585–594. [PubMed: 16586184]
- Dirig DM, Yaksh TL. Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. *J. Pharmacol. Exp. Ther*. 1995; 275:219–227. [PubMed: 7562553]

- Drew GM, Siddall PJ, Duggan AW. Mechanical allodynia following contusion injury of the rat spinal cord is associated with loss of GABAergic inhibition in the dorsal horn. *Pain*. 2004; 109:379–388. [PubMed: 15157699]
- Duguid, IC.; Smart, TG. Presynaptic NMDA Receptors. In: Van Dongen, AM., editor. *Biology of the NMDA Receptor*. Boca Raton, FL: CRC Press; 2009. p. 313-328.
- Dureja GP, Usmani H, Khan M, Tahseen M, Jamal A. Efficacy of intrathecal midazolam with or without epidural methylprednisolone for management of post-herpetic neuralgia involving lumbosacral dermatomes. *Pain Physician*. 2010; 13:213–221. [PubMed: 20495585]
- Eaton MJ. Cell and molecular approaches to the attenuation of pain after spinal cord injury. *J. Neurotrauma*. 2006; 23:549–559. [PubMed: 16629636]
- Eaton MJ, Martinez MA, Karmally S. A single intrathecal injection of GABA permanently reverses neuropathic pain after nerve injury. *Brain Res*. 1999; 835:334–339. [PubMed: 10415391]
- Erhardt S, Engberg G. Excitation of nigral dopamine neurons by the GABA(A) receptor agonist muscimol is mediated via release of glutamate. *Life Sci*. 2000; 67:1901–1911. [PubMed: 11043612]
- Faden AI, Lemke M, Simon RP, Noble LJ. N-methyl-D-aspartate antagonist MK801 improves outcome following traumatic spinal cord injury in rats: behavioral, anatomic, and neurochemical studies. *J. Neurotrauma*. 1988; 5:33–45. [PubMed: 3057216]
- Finnerup NB, Gyldensted C, Nielsen E, Kristensen AD, Bach FW, Jensen TS. MRI in chronic spinal cord injury patients with and without central pain. *Neurology*. 2003; 61:1569–1575. [PubMed: 14663044]
- Finnerup NB, Sorensen L, Biering-Sorensen F, Johannesen IL, Jensen TS. Segmental hypersensitivity and spinothalamic function in spinal cord injury pain. *Exp. Neurol*. 2007; 207:139–149. [PubMed: 17628539]
- Gracies JM, Nance P, Elovic E, McGuire J, Simpson DM. Traditional pharmacological treatments for spasticity. Part II: General and regional treatments. *Muscle Nerve Suppl*. 1997; 6:S92–S120. [PubMed: 9826984]
- Gwak YS, Hulsebosch CE. GABA and central neuropathic pain following spinal cord injury. *Neuropharmacology*. 2011a; 60:799–808. [PubMed: 21216257]
- Gwak YS, Hulsebosch CE. Neuronal hyperexcitability: a substrate for central neuropathic pain after spinal cord injury. *Curr. Pain Headache Rep*. 2011b; 15:215–222. [PubMed: 21387163]
- Hama A, Sagen J. Selective antihyperalgesic effect of [Ser1] histogranin on complete Freund's adjuvant-induced hyperalgesia in rats. *Pain*. 2002; 95:15–21. [PubMed: 11790463]
- Hama A, Sagen J. Behavioral characterization and effect of clinical drugs in a rat model of pain following spinal cord compression. *Brain Res*. 2007; 1185:117–128. [PubMed: 17935699]
- Hama A, Sagen J. Antinociceptive effects of the marine snail peptides conantokin-G and conotoxin MVIIA alone and in combination in rat models of pain. *Neuropharmacology*. 2009; 56:556–563. [PubMed: 19010337]
- Hama A, Woon Lee J, Sagen J. Differential efficacy of intrathecal NMDA receptor antagonists on inflammatory mechanical and thermal hyperalgesia in rats. *Eur. J. Pharmacol*. 2003; 459:49–58. [PubMed: 12505533]
- Hama AT, Sagen J. Cannabinoid receptor-mediated antinociception with acetaminophen drug combinations in rats with neuropathic spinal cord injury pain. *Neuropharmacology*. 2010; 58:758–766. [PubMed: 20035773]
- Hama AT, Siegan JB, Herzberg U, Sagen J. NMDA-induced spinal hypersensitivity is reduced by naturally derived peptide analog [Ser1]histogranin. *Pharmacol. Biochem. Behav*. 1999; 62:67–74. [PubMed: 9972847]
- Hammond DL, Washington JD. Antagonism of L-baclofen-induced antinociception by CGP 35348 in the spinal cord of the rat. *Eur. J. Pharmacol*. 1993; 234:255–262. [PubMed: 8387011]
- Hao JX, Xu XJ, Aldskogius H, Seiger A, Wiesenfeld-Hallin Z. Allodynia-like effects in rat after ischaemic spinal cord injury photochemically induced by laser irradiation. *Pain*. 1991; 45:175–185. [PubMed: 1652116]
- Heetla HW, Staal MJ, Kliphuis C, van Laar T. The incidence and management of tolerance in intrathecal baclofen therapy. *Spinal Cord*. 2009; 47:751–756. [PubMed: 19333246]

- Hentall ID, Hargraves WA, Sagen J. Inhibition by the chromaffin cell-derived peptide serine-histogranin in the rat's dorsal horn. *Neurosci. Lett.* 2007; 419:88–92. [PubMed: 17442490]
- Hentall ID, Sagen J. The alleviation of pain by cell transplantation. *Prog. Brain Res.* 2000; 127:535–550. [PubMed: 11142047]
- Herman RM, D'Luzansky SC, Ippolito R. Intrathecal baclofen suppresses central pain in patients with spinal lesions. A pilot study. *Clin. J. Pain.* 1992; 8:338–345. [PubMed: 1493344]
- Hwang JH, Yaksh TL. Effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. *Reg. Anesth.* 1997; 22:249–256. [PubMed: 9168217]
- Ishizawa Y, Furuya K, Yamagishi S, Dohi S. Non-GABAergic effects of midazolam, diazepam and flumazenil on voltage-dependent ion currents in NG108-15 cells. *Neuroreport.* 1997; 8:2635–2638. [PubMed: 9261841]
- Jeon Y. Cell based therapy for the management of chronic pain. *Korean J. Anesthesiol.* 2011; 60:3–7. [PubMed: 21359073]
- Knabl J, Witschi R, Hosl K, Reinold H, Zeilhofer UB, Ahmadi S, Brockhaus J, Sergejeva M, Hess A, Brune K, Fritschy JM, Rudolph U, Mohler H, Zeilhofer HU. Reversal of pathological pain through specific spinal GABAA receptor subtypes. *Nature.* 2008; 451:330–334. [PubMed: 18202657]
- Koizuka S, Obata H, Sasaki M, Saito S, Goto F. Systemic ketamine inhibits hypersensitivity after surgery via descending inhibitory pathways in rats. *Can. J. Anaesth.* 2005; 52:498–505. [PubMed: 15872129]
- Kroin JS, Bianchi GD, Penn RD. Spinal cord transection produces a long-term increase in GABAB binding in the rat substantia gelatinosa. *Synapse.* 1993; 14:263–267. [PubMed: 8248850]
- Kvarnstrom A, Karlsten R, Quiding H, Gordh T. The analgesic effect of intravenous ketamine and lidocaine on pain after spinal cord injury. *Acta Anaesthesiol. Scand.* 2004; 48:498–506. [PubMed: 15025615]
- Leem JW, Kim HK, Hulsebosch CE, Gwak YS. Ionotropic glutamate receptors contribute to maintained neuronal hyperexcitability following spinal cord injury in rats. *Exp. Neurol.* 2010; 224:321–324. [PubMed: 20211179]
- Lemaire S, Rogers C, Dumont M, Shukla VK, Lapierre C, Prasad J, Lemaire I. Histogranin, a modified histone H4 fragment endowed with N-methyl-D-aspartate antagonist and immunostimulatory activities. *Life Sci.* 1995; 56:1233–1241. [PubMed: 8614240]
- Lemaire S, Shukla VK, Rogers C, Ibrahim IH, Lapierre C, Parent P, Dumont M. Isolation and characterization of histogranin, a natural peptide with NMDA receptor antagonist activity. *Eur. J. Pharmacol.* 1993; 245:247–256. [PubMed: 8101490]
- Mao J, Gold MS, Backonja MM. Combination drug therapy for chronic pain: a call for more clinical studies. *J. Pain.* 2011; 12:157–166. [PubMed: 20851058]
- Mao J, Price DD, Hayes RL, Lu J, Mayer DJ, Frenk H. Intrathecal treatment with dextrophan or ketamine potently reduces pain-related behaviors in a rat model of peripheral mononeuropathy. *Brain Res.* 1993; 605:164–168. [PubMed: 8385540]
- Max MB, Byas-Smith MG, Gracely RH, Bennett GJ. Intravenous infusion of the NMDA antagonist, ketamine, in chronic posttraumatic pain with allodynia: a double-blind comparison to alfentanil and placebo. *Clin. Neuropharmacol.* 1995; 18:360–368. [PubMed: 8665549]
- McGowan MK, Hammond DL. Antinociception produced by microinjection of L-glutamate into the ventromedial medulla of the rat: mediation by spinal GABAA receptors. *Brain Res.* 1993; 620:86–96. [PubMed: 8104668]
- Menon-Johansson AS, Berrow N, Dolphin AC. G(o) transduces GABAB-receptor modulation of N-type calcium channels in cultured dorsal root ganglion neurons. *Pflugers Arch.* 1993; 425:335–343. [PubMed: 8309795]
- Millan MJ. The induction of pain: an integrative review. *Prog. Neurobiol.* 1999; 57:1–164. [PubMed: 9987804]
- Olivera BM, Teichert RW. Diversity of the neurotoxic Conus peptides: a model for concerted pharmacological discovery. *Mol. Interv.* 2007; 7:251–260. [PubMed: 17932414]
- Porreca F, Jiang Q, Tallarida RJ. Modulation of morphine antinociception by peripheral [Leu5]enkephalin: a synergistic interaction. *Eur. J. Pharmacol.* 1990; 179:463–468. [PubMed: 2364995]

- Raffa RB, Clark-Vetri R, Tallarida RJ, Wertheimer AI. Combination strategies for pain management. *Expert Opin. Pharmacother.* 2003; 4:1697–1708. [PubMed: 14521480]
- Rowbotham MC, Fields HL. The relationship of pain, allodynia and thermal sensation in post-herpetic neuralgia. *Brain.* 1996; 119:347–354. [PubMed: 8800931]
- Ruan H, Lemaire S. Interactions of histogranin and related peptides with dopamine D2 receptor in rat brain membranes. *Synapse.* 2001; 39:270–274. [PubMed: 11169776]
- Saulino M, Burton AW, Danyo DA, Frost S, Glanzer J, Solanki DR. Intrathecal ziconotide and baclofen provide pain relief in seven patients with neuropathic pain and spasticity: case reports. *Eur. J. Phys. Rehabil. Med.* 2009; 45:61–67. [PubMed: 19156022]
- Shapiro S. Neurotransmission by neurons that use serotonin, noradrenaline, glutamate, glycine, and gamma-aminobutyric acid in the normal and injured spinal cord. *Neurosurgery.* 1997; 40:168–176. discussion 177. [PubMed: 8971839]
- Shukla VK, Lemaire S, Dumont M, Merali Z. N-methyl-D-aspartate receptor antagonist activity and phencyclidine-like behavioral effects of the pentadecapeptide, [Ser1]histogranin. *Pharmacol. Biochem. Behav.* 1995; 50:49–54. [PubMed: 7700954]
- Siddall PJ, Molloy AR, Walker S, Mather LE, Rutkowski SB, Cousins MJ. The efficacy of intrathecal morphine and clonidine in the treatment of pain after spinal cord injury. *Anesth. Analg.* 2000; 91:1493–1498. [PubMed: 11094007]
- Siegan JB, Hama AT, Sagen J. Suppression of neuropathic pain by a naturally-derived peptide with NMDA antagonist activity. *Brain Res.* 1997; 755:331–334. [PubMed: 9175901]
- Taira T, Kawamura H, Tanikawa T, Iseki H, Kawabatake H, Takakura K. A new approach to control central deafferentation pain: spinal intrathecal baclofen. *Stereotac. Func. Neurosurg.* 1995; 65:101–105.
- Tallarida RJ. Interactions between drugs and occupied receptors. *Pharmacol. Ther.* 2007; 113:197–209. [PubMed: 17079019]
- Tallarida, RJ.; Murray, RB. *Manual of Pharmacological Calculations with Computer Programs.* New York, NY: Springer-Verlag; 1981.
- Tallarida RJ, Porreca F, Cowan A. Statistical analysis of drug-drug and site-site interactions with isobolograms. *Life Sci.* 1989; 45:947–961. [PubMed: 2677570]
- Towers S, Princiville A, Billinton A, Edmunds M, Bettler B, Urban L, Castro-Lopes J, Bowery NG. GABAB receptor protein and mRNA distribution in rat spinal cord and dorsal root ganglia. *Eur. J. Neurosci.* 2000; 12:3201–3210. [PubMed: 10998104]
- Vranken JH, Troost D, Wegener JT, Kruis MR, van der Vegt MH. Neuropathological findings after continuous intrathecal administration of S(+)-ketamine for the management of neuropathic cancer pain. *Pain.* 2005; 117:231–235. [PubMed: 16098665]
- Xu XJ, Hao JX, Seiger A, Wiesenfeld-Hallin Z. Systemic excitatory amino acid receptor antagonists of the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and of the N-methyl-D-aspartate (NMDA) receptor relieve mechanical hypersensitivity after transient spinal cord ischemia in rats. *J. Pharmacol. Exp. Ther.* 1993; 267:140–144. [PubMed: 8229741]
- Yaksh TL, Allen JW. Preclinical insights into the implementation of intrathecal midazolam: a cautionary tale. *Anesth. Analg.* 2004; 98:1509–1511. [PubMed: 15155298]
- Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol. Behav.* 1976; 17:1031–1036. [PubMed: 14677603]
- Zhang AL, Hao JX, Seiger A, Xu XJ, Wiesenfeld-Hallin Z, Grant G, Aldskogius H. Decreased GABA immunoreactivity in spinal cord dorsal horn neurons after transient spinal cord ischemia in the rat. *Brain Res.* 1994; 656:187–190. [PubMed: 7804836]

A.

B.

Figure 1.

Antinociceptive effect of intrathecally injected GABA receptor agonists in rats with a spinal cord injury. The horizontal axis is time in minutes post-intrathecal (i.t.) injection and the vertical axis is the withdrawal threshold in grams. The mean Pre-Injury ("PI") withdrawal threshold is 15 g (□). Rats were tested four weeks following spinal cord injury. Following baseline withdrawal threshold determination, rats were i.t. injected with either vehicle, (A) muscimol or (B) baclofen. N = 7/group. Data are expressed as mean ± S.E.M. **P* < 0.05 vs. baseline, # *P* < 0.05 vs. vehicle.

Figure 2.

Intrathecal injection of NMDA receptor antagonists in rats with a spinal cord injury. The horizontal axis is time in minutes post-i.t. injection and the vertical axis is the withdrawal threshold in grams. The mean Pre-Injury ("PI") withdrawal threshold is 15 g (□). Rats were tested four weeks following spinal cord injury. **A.** Time course following i.t. injection of either ketamine, [Ser¹]histogranin (SHG) or vehicle. Although withdrawal thresholds were increased following [Ser¹]histogranin injection, the increase was not statistically significant. **B.** Time course following i.t. injection of either MK-801 or vehicle. N = 3–7/group. Data are expressed as mean ± S.E.M. **P* < 0.05 vs. baseline, # *P* < 0.05 vs. vehicle.

A.

B.

Figure 3.

Effect of intrathecally injected combinations in rats with a spinal cord injury. The horizontal axis is the log-dose (μg) and the vertical axis is the percent maximum possible effect (MPE). The effects of the drugs at 30 min post-injection are shown. **A.** Dose-response curve (dashed line) of i.t. muscimol alone. Muscimol was combined with either ketamine or [Ser]¹-histogranin. Note that the effects of the highest doses of the muscimol combinations are less than 50% MPE. **B.** Dose-response curve (dashed line) of i.t. baclofen alone and baclofen (0.3 μg) combined with either ketamine or [Ser]¹histogranin. The solid line is the dose-response curve of the baclofen+ketamine combination. Since the percent MPE of the highest

dose of baclofen+[Ser¹]histogranin is 50%, only this dose is shown. N = 5–7/group. Data are expressed as mean $\pm$ S.E.M.

A.

B.

Figure 4.

Effect of pretreatment with the GABA_B receptor antagonist CGP 35348 on the antinociceptive effects of baclofen and the combination of baclofen+ketamine in rats with a spinal cord injury. **A.** Thirty μ g CGP 35348 was i.t. injected 30 min prior to injection of 1 μ g baclofen. Rats were tested 30 min after the second i.t. injection. There were four treatment groups: *i*) Vehicle/Vehicle, *ii*) CGP 35348/Vehicle, *iii*) Vehicle/Baclofen, *iv*) CGP 35348/Baclofen. * P < 0.05 vs. Vehicle/Vehicle, # P < 0.05 vs. Vehicle/Baclofen. **B.** Intrathecal injection of either 30 μ g CGP 35348 (CGP) or vehicle was followed 30 min later by i.t. injection of either the combination (0.3 μ g baclofen+100 μ g ketamine; Bac+Ket) or vehicle.

Rats were tested 30 min after the second i.t. injection. There were four treatment groups: *i*) Vehicle/Vehicle, *ii*) CGP/Vehicle, *iii*) Vehicle/Bac+Ket, *iv*) CGP/Bac+Ket. N = 6–7/group. Data are expressed as mean $\pm$ S.E.M. * P < 0.05 vs. Vehicle/Vehicle, # P < 0.05 vs. Vehicle/Bac+Ket.

Table 1

Antinociceptive effect of intrathecally injected drugs alone in rats with neuropathic SCI pain

	A₅₀ (95% C.L.)^a
Baclofen	0.3 (0.1)
Ketamine	>100
Muscimol	1.8 (1.4)
[Ser¹]histogranin	>10

^aA₅₀ is the 50 percent antinociceptive dose (95% Confidence Limits) in µg. (N = 4–7 rats).

Table 2

Effects of intrathecally injected combination drugs in neuropathic SCI rats

	Theoretical A ₅₀ ^a (95% C.L.)	Experimental A ₅₀ (95% C.L.)
<i>Muscimol with:</i>		
Ketamine	100.1 (83.2)	>100
[Ser¹]histogranin	11.2 (8.9)	28.5 (22.6)
<i>Baclofen with:</i>		
Ketamine	103.4 (43.3)	37.2 (10.0) ^b
[Ser¹]histogranin	10.3 (4.5)	12.6 (13)

^aThe A₅₀ (50 percent antinociceptive dose in µg) doses are total amounts of the constituents based on the proportion used in each combination (see Materials and methods). The effects of the combinations were determined 30 min post-injection. (N = 4–6 rats).

^b*P* < 0.05, synergistic: experimental A₅₀ < theoretical additive A₅₀.