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Modulation of spinal GABAergic analgesia by inhibition of chloride extrusion capacity in mice

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Abstract

Spinal GABA_A receptor modulation with agonists and allosteric modulators evokes analgesia and antinociception. Changes in KCC2 expression or function that occur after peripheral nerve injury can result in an impairment in the Cl[−] extrusion capacity of spinal dorsal horn neurons. This, in turn, alters Cl[−] mediated hyperpolarization via GABA_A receptor activation contributing to allodynia or hypersensitivity associated with nerve injury or inflammation. A gap in knowledge exists concerning how this loss of spinal KCC2 activity differentially impacts the analgesic efficacy or potency of GABA_A agonists and allosteric modulators. We utilized intrathecal drug administration in the tail flick assay to measure the analgesic effects of general GABA_A agonists muscimol and ZAPA, the α -subunit preferring agonist THIP and allosteric modulators of the benzodiazepine (midazolam) and neurosteroid (ganaxolone) class, alone, or in the presence of KCC blockade. Intrathecal muscimol, ZAPA, THIP midazolam and ganaxolone all evoked significant analgesia in the tail flick test. Co-administration of either agonists or allosteric modulators with DIOA (a drug that blocks KCC2) had no effect on agonist or allosteric modulator potency. On the other hand, the analgesic efficacy of muscimol and ZAPA and the allosteric modulator ganaxolone were markedly reduced while THIP and midazolam were unaffected. Finally, In the spared nerve injury (SNI) model, midazolam significantly reversed tactile hypersensitivity whilst ganaxolone had no effect. These results indicate that the KCC2-dependent Cl[−] extrusion capacity differentially regulates the analgesic efficacy of agonists and allosteric modulators at the GABA_A receptor complex.

Perspective—Our work suggests that drug discovery efforts for the treatment of chronic pain disorders should target benzodiazepine or α -subunit-containing sites at the GABA_A complex.

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Keywords

KCC2; GABA; THIP; ganaxolone; benzodiazepine; tail flick; neuropathic pain

Introduction

Targeting spinal ionotropic gamma-aminobutyric acid (GABA_A) receptors elicits analgesia in rodents⁴³ and humans^{26, 58}. The GABA_A agonist muscimol possesses antinociceptive activity against acute nociception^{1, 23, 37} and in the formalin model¹⁷. The δ subunit preferring agonist THIP similarly induces analgesia and antinociception in rats and mice^{25, 34, 35}. In addition to agonists, positive allosteric modulators, such as benzodiazepines, are effective in producing spinally-mediated analgesia^{6, 22, 63}. GABA_A-mediated inhibitory neurotransmission in both individual neurons and neuronal networks is modulated by cation-chloride cotransporter functional expression^{30, 49, 52, 54}. These cotransporters regulate neuronal Cl⁻ homeostasis. The K⁺-Cl⁻ cotransporter isoform 2 (KCC2) is largely responsible for Cl⁻ extrusion in mature CNS neurons^{7, 49}. Dysregulation in Cl⁻ homeostasis resulting from changes in functional KCC2 expression occurs in many CNS pathologies including epilepsy^{27, 45, 57} neuronal trauma^{46, 61} and chronic pain^{13, 51}. Because KCC2 maintains a low intracellular Cl⁻ concentration in CNS neurons, a prerequisite for the generation of Cl⁻-dependent, hyperpolarizing GABA_A-mediated responses^{30, 49, 56}, a disruption in functional KCC2 expression alters Cl⁻ homeostasis and can consequently lead to a reduction in efficacy of GABAergic inhibition.

In the spinal dorsal horn, KCC2 plays a key role in regulating nociceptive circuits. Hypomorphic KCC2 mice show altered sensitivity to tactile and noxious thermal stimuli⁶⁰ and a reduction in nociceptive thresholds in rats is observed when KCC2 expression is knocked down or pharmacologically inhibited¹³. Notably, reduced KCC2 expression or function has been implicated in the pathogenesis of neuropathic pain. Here, decreased functional KCC2 expression causes a depolarizing shift in GABA_A Cl⁻ reversal potential in lamina I/II neurons that leads to a reduction in GABAergic inhibitory efficacy in a subset of lamina I/II neurons^{13, 15, 51}. Despite these findings, there is strong evidence that modulation of spinal GABA_A receptors in the setting of peripheral nerve injury or inflammation^{4, 5, 32, 33} is still capable of producing antinociceptive or analgesic effects. Despite increasing knowledge of the analgesic and antinociceptive properties of subtype specific GABA_A receptor agonists and allosteric modulators, the influence of reduced KCC2 activity on the spinal analgesic efficacy and/or potency of these agonists and allosteric modulators remains unknown. However, this information is likely important for the development of novel analgesics that target the GABA_A receptor complex.

To directly address this question, we used the tail flick assay to measure the analgesic effects of GABA_A receptor agonists muscimol, ZAPA and THIP, and allosteric GABA_A modulators midazolam and ganaxolone. We utilized DIOA²¹ and furosemide⁴⁸ as inhibitors of KCC2. Muscimol and ZAPA³ are derived from the endogenous GABA_A receptor agonist GABA and target all GABA_A receptors without impacting GABA_B receptors. THIP preferentially targets δ -subunit containing GABA_A receptors² and activates a tonic current in spinal dorsal horn neurons⁸. Midazolam and ganaxolone¹¹ belong to two major classes of GABA_A allosteric modulators, benzodiazepines and neurosteroids, respectively. Benzodiazepines positively modulate both tonic and phasic currents in spinal dorsal horn neurons³⁸ while the effects of neurosteroids on these currents are unknown. Our results demonstrate that muscimol, ZAPA and ganaxolone lose analgesic efficacy, but not potency with KCC2 blockade while potency and efficacy of THIP and midazolam are unchanged. Moreover, midazolam significantly reverses tactile allodynia following

peripheral nerve injury while ganaxolone is without effect. These findings indicate that impaired Cl^- extrusion capacity resulting from decreased KCC2 activity reduces the analgesic efficacy of certain GABA_A receptor agonists and allosteric modulators.

Methods

Drugs

Muscimol, ZAPA, THIP, ganaxolone, and clonidine hydrochloride were purchased from Tocris (Ellisville, MO). DIOA and furosemide were purchased from Sigma-Aldrich (St. Louis, MO). Midazolam was purchased from USP. Stock solutions of muscimol, ZAPA, clonidine and midazolam were made in distilled H₂O. DIOA, furosemide, THIP and ganaxolone were made in 100% DMSO. DIOA was diluted in Ringer's solution containing 10% DMSO. All other drugs were diluted to final doses in Ringer's solution for injection. Final concentrations of DMSO in solutions used for intrathecal injection in the tail flick test were 10%. This concentration of DMSO had no effect on tail flick latency at any time point (baseline = 3.55 ± 0.33 s, 10 min post injection = 3.56 ± 0.40 s, 20 min post injection = 3.18 ± 0.25 s, 30 min post injection = 3.16 ± 0.13 sec, $n = 7$). Final concentrations of DMSO in intrathecal injections in neuropathic animals did not exceed 1%.

Animals

Male ICR mice (Harlan, 18–22g) were used for all studies. Animals were housed in a climate-controlled room on a 12-h light/dark cycle where food and water were available ad libitum. All animal procedures were approved by the Institutional Animal Care and Use Committee of the University of Arizona and were performed in accordance with the handling and use of laboratory animal guidelines of the International Association for the Study of Pain and National Institutes of Health.

Spared Nerve Injury (SNI)

Prior to surgery, all animals were assessed for mechanical withdrawal thresholds. SNI was performed by a lesion of two of the three terminal branches of the sciatic nerve (tibial and common peroneal nerves) leaving the remaining sural nerve intact as described previously⁹. Under isoflurane anesthesia (induction = 5%, maintenance = 1.7–2% isoflurane in room air), an incision was made on the lateral surface of the thigh and a section was made directly through the biceps femoris muscle exposing the sciatic nerve and its three terminal branches: the sural, common peroneal and tibial nerves. The tibial and common peroneal nerves were tightly ligated with 5.0 silk and cut distal to the ligation, removing ~ 2mm of the distal nerve stump. The sural nerve was left intact. The skin incision was closed with staples. Mice were monitored daily following surgery and were tested for development of neuropathic allodynia 7 and 10 days following surgery. Testing with administration of intrathecal drugs was done on day 10–14 after surgery. The neuropathic pain state in these mice lasts for at least 4 weeks and is isolated to the ipsilateral paw⁹.

Intrathecal injection

The intrathecal injection was done as previously described by Hylden and Wilcox²⁸ using a 25- μl Hamilton syringe with a 30 1/2-gauge needle. The injection was performed under brief (less than 3 min) isoflurane anesthesia (induction = 5%, maintenance = 1.7–2% isoflurane in room air) in a volume of 5 μl . With the pelvic girdle held to keep the spinal cord in place, the needle was inserted into the intervertebral space between the L5 and L6 spinal cord level. A reflexive flick of the tail was used as an indicator of accurate needle placement and also as a confirmation of drug injection.

Tail Flick Latency

Analgesia was measured using the tail immersion method²⁹. The mice were restrained with the tail extending out. The distal portion of the tail (2–3cm) was immersed in a water bath thermostatically controlled at 52°C ± 0.5. The tail withdrawal reaction time (in seconds) was initially recorded as the tail flick latency before drug administration and then recorded at 10, 20, 30, 45 and 60 min after the administration of the test drug. A cut off latency of 10 seconds was maintained to prevent tissue damage. All the drugs were administered intrathecally. Percent maximum possible effect was calculated as:

$$\%MPE = [(Test\ latency - Baseline\ latency) / (Cut\ off\ latency - Baseline\ latency)] * 100$$

Mechanical thresholds

Animals were placed in acrylic boxes with wire mesh floors and allowed to habituate for 1 hr followed by predrug mechanical thresholds recording. Using the up-down method¹², calibrated von Frey filaments (Stoelting, Wood Dale, IL) were used for mechanical stimulation of the plantar surface of the left hindpaw and withdrawal thresholds were calculated.

Statistics

All data are presented as Mean ± S.E.M unless otherwise stated. Graph plotting and statistical analysis were performed using Graphpad Prism Version 5.03 (Graph Pad Software, Inc. San Diego, CA, USA). Variable slope (4 parameters) nonlinear regression was used for dose response curves analysis. Measures of dose by time were done by two-way ANOVA with Bonferroni post-hoc test in tail flick tests. For analysis of mechanical thresholds in neuropathic animals, mechanical thresholds are expressed as log of gram force and analyzed by one-way ANOVA with Dunnett post-test (as described previously⁴⁰) where comparisons were made to baseline mechanical thresholds at 10–14 days after SNI. A priori level of significance at 95% confidence level was considered at $p < 0.05$.

Results

We first assessed the ability of GABA_A receptor agonists and allosteric modulators to evoke analgesia in the tail flick test following intrathecal injection. The GABA_A agonists muscimol (Fig 1A), THIP (Fig 1B) and ZAPA (Fig 1C) each caused significant analgesia in the tail flick test peaking at 0.3, 10 and 3 µg doses, respectively. Tail flick latencies returned to baseline levels within 60 min. Peak effects were observed at 10 or 20 min post injection. We then retested each of these agonists with co-intrathecal administration of the KCC2 inhibitor DIOA (10 µg). In the presence of DIOA, peak analgesic effects were also observed at 10 or 20 min post injection. The GABA_A allosteric modulators midazolam (Fig 1D) and ganaxolone (Fig 1E) and the α₂ adrenergic receptor agonist, clonidine (Fig 1F), also each caused significant analgesia in the tail flick test peaking at 10, 10 and 2.67 µg doses, respectively. Tail flick latencies returned to baseline levels within 60 min for each compound. We then retested each of these agonists with co-intrathecal administration of the KCC2 inhibitor DIOA (10 µg). Again, peak effects were observed at 10 or 20 min post injection in the presence and absence of DIOA. We reasoned that blockade of KCC2 with DIOA might alter either efficacy or potency (or both) for GABA_A agonists or allosteric modulators in the tail flick test. We further anticipated that analgesic efficacy and potency of clonidine would be unaffected by KCC2 perturbation because this drug does not act on the GABA_A receptor complex. In order to establish the analgesic efficacy and potency of the different agonists and allosteric modulators alone and in the presence of DIOA, dose response curves were constructed and evaluated. The dose response curves were graphed

using the sum of the percent maximal effect (%MPE) at 10 and 20 min time points where peak analgesia was noted for all GABA_A agonists and allosteric modulators used. Due to the steep and clear inverted U-shaped nature (in agreement with our previous observations and modeling studies^{5, 18}) of the dose response curves obtained, the curves for all compounds used were fitted with a fixed $E_{\max}$ (a measure of efficacy) based on the maximally effective doses listed above. Thus $E_{\max}$ values for the different agonists and allosteric modulators could not be found and evaluated through this method. Dose response curves were therefore evaluated using the ED₅₀s (a measure of potency) and confidence intervals derived from these curves. A comparison of the ED₅₀s and of the different agonists and allosteric modulators alone and in the presence of DIOA (Table 1) showed an overlap in confidence intervals for the different agonists and allosteric modulators alone and with DIOA present indicating that potency was unchanged by DIOA treatment. This result also illustrates that DIOA is not acting as a competitive antagonist at GABA_A receptors in this assay.

From the plotted %MPE data from figure 1, it was evident that the major effect of DIOA was a reduction in analgesic efficacy (e.g. reduced $E_{\max}$) for some agonists or allosteric modulators. Thus, we compared the peak effect responses for each compound $\pm$ DIOA. For the general GABA_A agonists muscimol (Fig 2A) and ZAPA (Fig 2B), DIOA co-treatment significantly reduced analgesic efficacy at either 10 or 20 min post injection indicating a reduction in analgesic efficacy in the presence of KCC2 blockade. In contrast, the δ -subunit preferring agonist THIP failed to lose analgesic efficacy with KCC2 blockade (Fig 2C). The benzodiazepine GABA_A allosteric modulator, midazolam, showed no loss of analgesic efficacy with KCC2 blockade (Fig 2D) whereas the neurosteroid site ligand, ganaxolone did (Fig 2E). As expected, there was no change in analgesic efficacy in the presence of DIOA for clonidine (Fig 2F). To further support the reduced analgesic efficacy observed with DIOA treatment, we asked if similar effects would be observed with the structurally dissimilar KCC2 inhibitor furosemide⁴⁷. Furosemide (30 μ g) did not influence the effects of THIP (Fig 3A) but reduced analgesic efficacy for ganaxolone (Fig 3B) consistent with findings with DIOA. Importantly, neither DIOA at 10 μ g (%MPE at 10 min = $0.83 \pm 3.28\%$ and 20 min = $0.63 \pm 4.21\%$) nor furosemide at 30 μ g (%MPE at 10 min = $6.66 \pm 8.94\%$ and 20 min = $1.75 \pm 4.28\%$) alone had an effect in the tail flick test.

Reduced chloride extrusion capacity is thought to underlie neuropathic allodynia following peripheral nerve injury^{15, 52, 54}. This dysregulation in chloride extrusion capacity has been shown to occur via changes in functional KCC2 expression^{13, 31}. Because we observed differential effects of GABA_A allosteric modulator analgesic efficacy with KCC2 blockade, we hypothesized that midazolam would retain anti-allodynic effects in the spared nerve injury model while ganaxolone would lack anti-allodynic effects. Using doses of midazolam and ganaxolone that were efficacious in the tail flick test under unperturbed KCC2 activity, midazolam significantly reversed neuropathic allodynia observed in the spared nerve injury model (Fig 4A) whereas ganaxolone had no effect (Fig 4B). This result confirms our hypothesis and suggests that the analgesic efficacy of allosteric modulators that differentially target the GABA_A receptor complex are under control of KCC2-mediated Cl⁻ extrusion capacity.

Discussion

Although there is ample evidence for the analgesic and antinociceptive properties of GABA_A receptor agonists and allosteric modulators at the spinal level, the effect of loss of Cl⁻ extrusion capacity on their analgesic and antinociceptive properties has hitherto been unexplored. Hence, the primary findings of the current work are that loss of Cl⁻ extrusion capacity does not affect the potency (ED₅₀) of either agonists or allosteric modulators of the GABA_A receptor complex, but, rather, modulates the efficacy ($E_{\max}$) of non-selective

GABA_A agonists and neurosteroid-derived allosteric modulators. Specifically, the agonists muscimol and ZAPA and the allosteric modulator ganaxolone lose analgesic efficacy whereas the δ -subunit preferring agonist THIP and the allosteric modulator midazolam maintain efficacy when Cl⁻ extrusion capacity is inhibited. Importantly, our findings illustrate that spinal midazolam attenuates tactile hypersensitivity following peripheral nerve injury, which reduces spinal KCC2 expression¹³, whilst ganaxolone is without effect. Thus, in clinical situations where spinal functional Cl⁻ extrusion capacity is thought to be diminished, targeting spinal GABA_A receptors containing the δ -subunit and/or sensitive to benzodiazepines is likely to represent a relevant therapeutic approach.

In spinal dorsal horn neurons, GABA_A receptor agonists have been demonstrated to attenuate glutamate-induced and/or spontaneous excitability^{14, 65} and GABA_A agonists and/or allosteric modulators applied intrathecally increase nociceptive thresholds in rodents^{1, 17, 23}. Consistent with these results, our present findings indicate that the GABA_A agonists muscimol, THIP and ZAPA and allosteric modulators midazolam and ganaxolone produce analgesia in the mouse tail flick test. An important question in terms of GABA_A-based therapeutics for the treatment of pain in humans arises from our current understanding of the role of KCC2 in modulating GABA_A-mediated currents in preclinical models. KCC2 expression is decreased in the spinal dorsal horn following peripheral nerve injury and after peripheral inflammation or injury^{13, 39, 41, 44, 64}. This change in KCC2 expression is thought to decrease GABA_A-dependent inhibition in a subset of dorsal horn neurons and has been proposed as a primary mechanism for the generation of allodynia in preclinical neuropathic pain models^{13, 15, 52, 54}. However, it is also clear that benzodiazepine class allosteric modulators maintain efficacy for reducing neuropathic allodynia and/or inflammation-induced hyperalgesia^{4, 5, 32, 33}. These findings suggest that altered functional KCC2 expression may differentially modulate the analgesic efficacy of GABA_A agonists or allosteric modulators⁵⁴. Our findings strongly support this conclusion and point to specific therapeutic avenues for the generation of effective spinal analgesics that target the GABA_A receptor complex.

Heteropentameric GABA_A receptors composed of multiple subunits (α 1–6, β 1–3, γ 1–3, δ , ϵ , θ and ρ) mediate both phasic (synaptic) and tonic (extrasynaptic) inhibition^{19, 20}. The transient activation of phasic GABA_A receptors composed of two α subunits combined with two β and a γ subunit produces phasic inhibition mediating fast GABAergic inhibitory transmission. Tonic inhibition is predominately generated by the persistent action of extrasynaptic receptors (composed of two α subunits combined with two β and a δ subunit or γ 2 subunit) by ambient GABA. These GABA_A receptors are based on high affinity isoforms (e.g. α 6 β $\times$ δ , α 4 β $\times$ δ and α 5 β $\times$ γ 2) with slow desensitization kinetics^{19, 20}. Muscimol and ZAPA do not pharmacologically distinguish GABA_A receptors of the tonic and phasic types²⁰; however, THIP preferentially acts at receptors of the tonic type⁴². Electrophysiological studies have shown that THIP exhibits superagonist activity at α 4 β 3 δ containing receptors^{2, 10} attributed to its efficacy at tonic receptors (Mortensen et al, 2010). Our current work shows that THIP, unlike muscimol and ZAPA, produces analgesia in the tail flick test which is unaffected by KCC2 inhibition. This suggests that reduced Cl⁻ extrusion capacity does not modulate THIP's analgesic activity. In agreement with our results, others have demonstrated the antinociceptive effects of THIP in preclinical models⁸ where KCC2 expression is at least transiently decreased⁴⁴. We therefore propose that selective activation of tonic GABA_A receptors in the setting of decreased Cl⁻ extrusion capacity may represent a viable approach for pharmacological intervention at the spinal level in chronic pain states. Further electrophysiological work will be needed to test this hypothesis in more detail.

Ganaxolone is a high affinity synthetic neurosteroid with positive allosteric modulatory effects at the GABA_A receptor complex. It has been shown to have anticonvulsive effects in preclinical models of seizure^{55, 59}. Ganaxolone potentiates GABA_A-evoked Cl⁻ currents with similar efficacy and potency as allopregnanolone at recombinant human $\alpha 1\beta 2\gamma 2$ and $\alpha 2\beta 2\gamma 2$ GABA_A receptors¹¹. Classical benzodiazepines, such as midazolam, target GABA_A receptors composed of $\alpha 1$, $\alpha 2$, $\alpha 3$ or $\alpha 5$ subunits together with β and $\gamma 2$ subunits. Benzodiazepines potentiate GABA_A channel opening by increasing agonist affinity³⁶. Benzodiazepines are known to elicit spinal analgesia and this effect is maintained in neuropathic and inflammatory pain models. It has recently been shown that midazolam enhances a tonic GABA_A current in substantia gelatinosa neurons³⁸ indicating that spinal, tonic GABA_A receptors are benzodiazepine sensitive. Here we show that midazolam maintains analgesic efficacy in the face of pharmacological perturbation of Cl⁻ extrusion capacity whereas ganaxolone efficacy is strongly reduced. Moreover, in the SNI model, midazolam effectively reverses mechanical allodynia while ganaxolone is without effect at doses that are efficacious in the tail flick test without Cl⁻ extrusion capacity manipulation. These findings, combined with data from GABA_A agonists, suggest that ganaxolone (and other neurosteroids) may lack effects on presynaptic and/or tonic GABA_A receptors in the spinal dorsal horn.

A major unresolved question from these studies is whether the primary site of action of GABAergic agonists and allosteric modulators employed here, in the presence and absence of Cl⁻ extrusion capacity blockade, is pre- or post-synaptic. THIP and midazolam maintained analgesic efficacy even in the presence of Cl⁻ extrusion capacity inhibition and midazolam reversed neuropathic allodynia in the SNI model. Recent findings concerning the nature of spinal analgesia induced by benzodiazepines suggest that this action is mediated primarily by $\alpha 2$ -containing GABA_A subunits³² and that these receptors reside on presynaptic terminals of primary afferents in the spinal dorsal horn⁶². Interestingly, THIP also has a strong effect on primary afferents and stimulates primary afferent depolarization^{16, 50}. Because primary afferents are devoid of KCC2 expression⁵³, it is tempting to speculate that the insensitivity of benzodiazepine- and THIP-induced analgesia to Cl⁻ extrusion capacity inhibition is due to an action of these compounds on GABA_A receptors expressed by primary afferents. If this is the case, analgesia mediated by these compounds is unlikely to be influenced by NKCC1 blockade because furosemide⁴⁷, which also inhibits NKCC1, also did not influence THIP-induced analgesia. As discussed above, THIP and benzodiazepines also have effects on postsynaptic tonic receptors in the spinal dorsal horn, hence, electrophysiological studies will be required to clearly define pre- vs. post-synaptic activities of these compounds in the presence of Cl⁻ extrusion capacity blockade.

There are several limitations to the present study that we wish to acknowledge. First, we have utilized DIOA and furosemide to influence Cl⁻ extrusion capacity in the spinal cord. While these compounds inhibit KCC2, they also have effects on other co-transporters: DIOA inhibits all KCCs and furosemide inhibits NKCC1 and 2 at least as potently as it influences KCCs⁴⁷. Second, we have previously observed dose-limiting effects of spinal benzodiazepines in preclinical neuropathic pain models as demonstrated by inverted U-shaped anti-allodynic dose response effects⁵. Similar results were obtained in the present studies at high doses suggesting that Cl⁻ extrusion capacity is insufficient to maintain GABA_A mediated analgesia under conditions of intense receptor activation, a suggestion supported by recent modeling studies¹⁸. Although doses of compounds higher than those shown in this study caused motor impairment, again, inverted U-shaped effects in the tail-flick test were observed at doses shown here strongly suggesting that analgesic effects were not due to motor impairment. Finally, although drugs were given intrathecally, we cannot completely rule out an effect on higher brain centers; however, GABA_A agonist injection

into the brainstem causes a decrease, not increase, in tail flick latency suggesting that the observed effects are largely due to a spinal action ²⁴.

In conclusion, the present findings provide direct evidence for differential modulation of spinal GABAergic analgesia when Cl⁻ extrusion capacity is reduced via KCC2 blockade. Our findings indicate that while potency remains unchanged, muscimol, ZAPA and ganaxolone demonstrate marked declines in analgesic efficacy. On the other hand, THIP and midazolam retain full efficacy when KCC2 is blocked. Because KCC2 expression is decreased in the spinal dorsal horn in preclinical neuropathic pain models ^{13, 39, 52, 54}, these results have potential implications for the generation and clinical testing of GABA_A agonists and allosteric modulators for the treatment of chronic pain disorders.

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Figure 1.

Dose response curves for intrathecal GABA_A agonists and allosteric modulators with and without DIOA treatment in the tail flick test.

Log doses of drug in grams are shown on x-axis while sum of %MPE for 10 and 20 min time points are shown on y-axis. Dose response curves are shown with agonist or allosteric modulators $\pm$ DIOA. ED₅₀s calculated from these curve fits (log(agonist) vs. response – Variable slope (four parameters)) are shown in Table 1.

Figure 2.

Peak-effect responses of intrathecal GABA_A agonists and allosteric modulators with and without DIOA treatment in the tail flick test.

Maximal effective doses for each agonist or allosteric modulator are shown $\pm$ DIOA expressed as %MPE over a 60 min time course. DIOA reduced analgesic efficacy for agonists muscimol (A) and ZAPA (B) but did not influence THIP (C). DIOA did not influence midazolam (D) efficacy but did reduce ganaxolone efficacy (E). Clonidine (F) was unaffected by DIOA. Two-way ANOVA with Bonferroni post-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 3.

Peak-effect responses for intrathecal THIP and ganaxolone with and without furosemide treatment.

Furosemide had no effect on THIP (A) analgesic efficacy but significantly reduced ganaxolone analgesia (B). Two way ANOVA with Bonferroni post-test. ** $p < 0.01$, *** $p < 0.001$.

Figure 4.

Effect of intrathecal midazolam and ganaxolone on spared nerve injury-induced neuropathic allodynia.

SNI surgery was performed and mice were tested for mechanical allodynia on the ipsilateral hindpaw 10–14 days post surgery. (A) intrathecal injection of midazolam significantly reversed mechanical allodynia in the mouse SNI model whereas ganaxolone (B) was without effect. One way ANOVA with Dunnet post-test comparing to baseline. * $p < 0.05$, *** $p < 0.001$.

Table 1

ED₅₀ of intrathecal GABA_A agonists and allosteric modulators with and without DIOA (10 µg) treatment in the mouse tail flick test. No significant changes in ED₅₀ were observed with DIOA co-treatment.

Drug	ED ₅₀	95% CI	ED ₅₀	95% CI
	Drug Alone		+ DIOA (10µg) ³	
Muscimol	83.6 ng (0.42 nmoles)	48.6 ng to 144 ng	4.70 ng (0.024 nmoles)	0.23 ng to 94.4 ng
THIP	1.05 µg (5.9 nmoles)	671 ng to 1.63 µg	776 ng (4.4 nmoles)	484 ng to 1.24 µg
ZAPA	3.04 µg (12.4 nmoles)	1.25 µg to 7.40 µg	560 ng (2.3 nmoles)	144 ng to 2.17 µg
Midazolam	695 ng (2.1 nmoles)	501 ng to 963 ng	1.33 µg (4.1 nmoles)	508 ng to 3.50 µg
Ganaxolone	3.22 µg (9.7 nmoles)	2.04 µg to 5.08 µg	3.74 µg (11.2 nmoles)	1.64 µg to 8.50 µg
Clonidine	203 ng (0.76 nmoles)	45.9 ng to 894 ng	798 ng (3.0 nmoles)	560 ng to 1.14 µg