Published in final edited form as:

Anesth Analg. 2011 April ; 112(4): 971–976. doi:10.1213/ANE.0b013e31820f2a16.

The Effects of Intrathecal and Systemic Gabapentin on Spinal Substance P Release

Toshifumi Takasusuki, M.D., Ph.D. and

Department of Anesthesiology, Dokkyo Medical University, School of Medicine, Tochigi, Japan

Tony L. Yaksh, Ph.D.

Department of Anesthesiology, University of California, San Diego, La Jolla, California

Abstract

Background—Gabapentin binds at the extracellular $\alpha 2\delta 1$ subunit of voltage sensitive calcium channels. Some voltage sensitive calcium channels regulate substance P release from small primary afferents. We sought to determine *in vivo* if spinal and/or systemic gabapentin at antihyperalgesic doses will attenuate substance P release.

Methods—Rats prepared with chronic intrathecal (IT) catheters received IT vehicle or gabapentin 10 min before intraplantar formalin (5%, 50 μ l) injection. For systemic studies, vehicle or gabapentin was delivered intraperitoneally (IP) 15 min before formalin injection. In separate groups of rats, to assess the effect of IT or IP gabapentin upon formalin-evoked substance P release, animals received similar treatment for assessment of flinching, but underwent transcardial perfusion with 4% paraformaldehyde 10 min after the formalin injection. Substance P release was determined by the incidence of neurokinin 1 receptor (NK1r) internalization in the ipsilateral and contralateral superficial dorsal horn in immunofluorescent stained tissues.

Results—Unilateral intraplantar formalin evoked biphasic hindpaw flinching. IT gabapentin (100 and 200 μ g) and IP gabapentin (100 and 200 mg/kg) resulted in a dose-dependent reduction in phase 2, but not phase 1 flinching as compared to vehicle-treated rats. Intraplantar formalin resulted in NK1r internalization in the ipsilateral, but not contralateral, superficial dorsal horn. IT gabapentin (200, but not 100 μ g) and IP gabapentin (200, but not 100 mg/kg) significantly reduced ipsilateral NK1r internalization as compared to vehicle-treated control. Importantly, internalization evoked by IT substance P was not blocked by IT gabapentin.

Corresponding Author: Tony L. Yaksh, Ph.D. Department of Anesthesiology, University of California, San Diego 9500 Gilman Drive, La Jolla, California 92013-0818 tyaksh@ucsd.edu; Phone: 619-543-3597; Fax: 619-543-6070.

DISCLOSURES Name: Toshifumi Takasusuki, M.D., Ph.D. **Contribution:** Conduct of study and data analysis

Name: Tony L. Yaksh, Ph.D.

Contribution: Study design and manuscript preparation

RECUSE NOTE Tony L. Yaksh is one of the Section Editors of Pain Mechanisms for the Journal. This manuscript was handled by Quinn H. Hogan, the second Section Editor of Pain Mechanisms, and Dr. Yaksh was not involved in any way with the editorial process or decision.

The authors declare no conflicts of interest.

Preliminary data was presented as a poster at the American Society of Anesthesiology Annual meeting, San Diego. 2010.

Reprints will not be available from the authors.

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Conclusion—Systemic and spinal gabapentin have an acute inhibitory effect on the release of substance P from small primary afferents and a concurrent effect upon the initiation of facilitated pain states.

Introduction

Gabapentin has a prominent antihyperalgesic action after either systemic or spinal (intrathecal: IT) delivery.¹⁻⁵ Previous work emphasized that the molecule binds with high affinity to the $\alpha 2\delta 1$ extracellular auxiliary subunit of voltage sensitive calcium channels (VSCCs), particularly the N- and L-, but not T-types.^{6,7} Point mutation studies of the $\alpha 2\delta 1$ protein showed that such changes had no effect upon calcium channel function, but completely prevented the binding and actions of gabapentin.⁸ Although the analgesic efficacy of gabapentin in various facilitated pain states is well known, its mechanism of action remains uncertain. Given the structural link between $\alpha 2\delta 1$ and the pore forming elements of the VSCCs and the presence of $\alpha 2\delta 1$ in dorsal root ganglion (DRG)⁹, a prevalent hypothesis is that gabapentin regulates afferent terminal transmitter release through regulation of calcium channel function. However, this hypothesis is complex. Thus, while an acute regulation by gabapentin of DRG calcium currents has been reported¹⁰⁻¹², gabapentin had only modest effects upon the frequency and amplitude of excitatory postsynaptic currents in superficial dorsal horn neurons.¹³ That work suggests that ongoing presynaptic neurotransmitter release is not regulated by this drug. Direct measurement of release in spinal slices has shown that gabapentin can diminish release of glutamate and several peptides,^{14,15} though with the *caveat* that the effects appear limited to conditions associated with prior sensitization or lengthy drug exposures.^{16,17} Other work has emphasized that gabapentin's actions may be dependent upon the upregulation of $\alpha 2\delta 1$ as occurs after nerve injury⁹ or may alter calcium channel trafficking.¹⁸ Such hypotheses imply the likelihood that the antihyperalgesic actions of gabapentin are limited to events that may require exposures of hours to days. Yet, it is evident from the acute antihyperalgesic effects of gabapentin *in vivo* that other acute mechanisms must be in play. The present experiments aimed to determine the effects of gabapentin *in vivo* on the spinal release of substance P evoked by intraplantar formalin injection. As dorsal horn substance P is primarily contained in small TRPV1 (+) primary afferents, changes in its release reflect an effect upon the releasing function of that small afferent terminal, in contrast to a transmitter such as glutamate which may arise from many neurons and even glia. In the present work, substance P release was examined *in vivo* by assessing the internalization of the neurokinin/receptor (NK1r) in the superficial dorsal horn produced by unilateral intraplantar formalin injection. NK1rs are densely located on lamina I projection neurons, but are largely absent from lamina II neurons.^{19,20} Finally, we sought to compare the effects of gabapentin given IT and intraperitoneally (IP) at equianalgesic doses. Previous work with morphine interestingly demonstrated that internalization was blocked at analgesic doses after IT delivery, but supra-analgesic doses after systemic delivery.²¹ As intraplantar formalin injection evokes a well defined biphasic flinching response, the *in vivo* activity of gabapentin on release and pain behavior can be compared.

Methods

Animals

Male Holtzman Sprague-Dawley rats (250-300g; Harlan Indianapolis, IN, USA) were individually housed and maintained on a 12-h light/dark cycle with testing occurring only during the light cycle. Food and water were available *ad libitum*. Animal care was in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes

of Health publication 85-23, Bethesda, MD, USA) and as approved by the institutional Animal Care and Use Committee of the University of California, San Diego.

Intrathecal catheter implantation

Rats were implanted with a single IT catheters for drug delivery.^{22,23} In brief, rats anesthetized with isoflurane in an air/oxygen mixture (1:1) were implanted with a polyethylene (OD 0.36mm) catheter passed through the cisternal membrane into the IT space to the level of the L2-L3 spinal segments (approximately 8.5 cm) and externalized on top of the head. Animals were given 5ml of lactated Ringer's solution subcutaneously and recovered under a heat lamp. Rats without motor deficit were allowed to recover for 5-7 days before study.

Formalin-induced paw-flinching behavior

To assess formalin-evoked flinching, a metal band was placed around the left hindpaw of the animal and the rat allowed to acclimate 30 min before experimental manipulation. At time 0, formalin (5%, 50 μ L) was delivered into the left hindpaw. Immediately after the formalin injection, rats were placed into the test chamber and nociceptive behavior (flinching and shaking of the injected paw) was quantified by an automatic flinch counting device (UARDG, Department of Anesthesiology, University of California, San Diego).²⁴ Flinches were counted in 1-min intervals for 60 min. The data are expressed as total number of flinches observed during phase 1 (0-10 min) and phase 2 (11-60 min).

Drugs and delivery

Gabapentin (Sigma Chemical, St Louis, MO, USA) was dissolved in 0.9% saline to be delivered in a volume of 10 μ L for IT delivery or to be delivered IP in a volume of 1.5 mL/kg. Drugs were administered IT 10 min or IP 15 min before the intraplantar delivery of formalin (5%, 50 μ L).

Effects of intrathecal gabapentin on NK1r internalization induced by exogenous substance P

To exclude the possibility that gabapentin directly blocked the NK1r internalization mechanism, rats were administered IT saline or gabapentin before IT substance P (30nmol). Thirty min later rats were killed and fixed. Spinal NK1r immunoreactive neurons, with or without NK1r internalization, were counted.

Tissue preparation and immunocytochemistry

For tissue harvest (10 min after intraplantar formalin), rats were deeply anesthetized with isoflurane and transcardially perfused with 0.9% NaCl followed by 4% paraformaldehyde in 0.1M sodium phosphate buffer (PBS), pH 7.4. The lumbar cord was removed and postfixed overnight. After cryoprotection in 30% sucrose, coronal sections were taken using a sliding microtome (30 μ m). To assess NK1r expression in the spinal cord dorsal horn, sections were incubated with a rabbit anti-NK1r polyclonal antibody (1:3000 in 0.01M PBS containing 10% normal goat serum and 10% Triton X-100; Advanced Targeting Systems (San Diego, CA, USA) overnight at room temperature. After a rinse in PBS, sections were incubated for 120 min at room temperature in a goat anti-rabbit secondary antibody conjugated with Alexa Fluor 488 (1:1000 in same buffer; Invitrogen, Eugene, OR, USA). All sections were rinsed, mounted on glass slides and coverslipped with ProLong mounting medium (Fisher Scientific, Pittsburgh, PA, USA).

Quantification of NK1r internalization

NK1r internalization was counted using an Olympus BX-51 fluorescence microscope (Olympus Optical, Tokyo, Japan) and a 60× oil-immersion objective lens and internalization assessed according to the standard of previous reports.^{19,25} The filter set was Omega Optical XF100-2 Green Bandpass Filter. Neuronal profiles that had 10 or more endosomes were considered to have internalized NK1rs. The total number of NK1r immunoreactive neurons in lamina I/II, with and without NK1r internalization, was counted and taken to calculate the fraction of cells showing internalization. Counting was done without knowledge of treatments. Mean counts from 2-5 sections per segment were used as representative counts for a given animal.²⁶ Images were taken using Magna FIRE SP and processed by adobe Photoshop CS4. Nomenclature for drugs and receptors conforms with the guide to receptors and channels of the *British Journal of Pharmacology*.²⁷

Statistical analysis

Changes in paw-flinching behavior were analyzed using one-way ANOVA for phase 1 and 2 with Tukey's *post hoc* tests performed for pairwise comparisons. Analysis of NK1r internalization data consisted of one-way or two-way ANOVAs with Tukey's *post hoc* analysis. Significance was defined as $P < 0.05$.

Results

Effects of intrathecal and intraperitoneal gabapentin on formalin evoked NK1r internalization

NK1r immunoreactivity was typically observed outlining the cytosolic membrane in many superficial dorsal horn neurons (Fig 1B). Unilateral intraplantar injection of formalin (5%, 50 μ l) produced a robust NK1r internalization in ipsilateral superficial dorsal horn, (Figure 1A, 2C) but not contralateral dorsal horn (ipsilateral L4: $26 \pm 7\%$, L5: $58 \pm 6\%$, L6: $59 \pm 6\%$, contralateral L4: $10 \pm 4\%$, L5: $4 \pm 3\%$, L6: $16 \pm 5\%$, $P < 0.05$). IT gabapentin (200 μ g but not 100 μ g) showed a prominent reduction in the ipsilateral L5 and L6 increase in formalin-evoked internalization ($P < 0.05$) (Figure 2C). IP gabapentin (200 mg/kg, but not 100mg/kg) significantly reduced the formalin-induced NK1r internalization at L5 and L6 levels of the superficial lumbar spinal cord ($P < 0.05$) (Figure 3C).

Effects of intrathecal gabapentin on NK1r internalization induced by exogenous substance P

IT substance P (30nmol) produced widespread NK1r internalization at L4-L6 levels of spinal cord lamina I compared with IT saline ($P < 0.05$) (Figure 4A-C). IT gabapentin (200 μ g), a dose that completely blocked formalin-induced NK1r internalization, did not affect exogenous substance P-induced NK1r internalization (Figure 4B, 4D).

Effects of intrathecal and intraperitoneal gabapentin on formalin evoked flinching behavior

Intraplantar formalin resulted in a robust biphasic incidence of flinching over a 60-min period. Pretreatment with IT gabapentin (100 and 200 μ g) resulted in a dose-dependent suppression of phase 2 flinching with no effect on phase 1 as compared to vehicle-treated control (Figure 2A, 2B). Similarly, after IP delivery, gabapentin (100 and 200 mg/kg) diminished phase 2, but not phase 1 as compared to vehicle control (Figure 3A, 3B). Importantly, IT gabapentin at neither 100 nor 200 μ g produced adverse effects on behavior or motor function as compared to vehicle control (Table 1). IP 100 and 200mg/kg gabapentin was observed to be associated with reduced spontaneous activity in some rats. No other motor effects were noted.

Discussion

As reviewed, binding to the $\alpha 2\delta 1$ subunit associated with VSCCs is likely essential for the spinal antihyperalgesic actions of gabapentin and its analogues.²⁸ The association of $\alpha 2\delta 1$ subunits with VSCCs in DRGs, along with the likelihood that one or more of these VSCCs may be instrumental in mediating depolarization-evoked transmitter release raise the possibility that gabapentin may regulate release from primary afferents.²⁹

Afferent substance P release

The present results indeed indicate that gabapentin given either IT or IP exert a potent, short latencied effect *in vivo* upon small afferent-evoked substance P release. Previous work showed that IT gabapentin reduced formalin-induced release of glutamate in spinal dorsal horn³⁰ and systemic gabapentin reduced spinal glutamate release after a visceral irritant.³¹ However, unlike the present work with substance P, the effects of gabapentin on spinal glutamate release cannot necessarily be ascribed to primary afferents given the ubiquitous nature of spinal glutamate distribution. The present effects of gabapentin on substance P release are in accordance with recent work in which we showed that substance P release in spinal dorsal horn can be attenuated *in vivo* by IT N-, but not T- or L-type calcium channel blockers.³²

The use of NK1r internalization as an index of primary afferent substance P release has been validated by several criteria and reviewed previously.^{21,33} An important *caveat* is the concern that the agent has a direct effect upon the postsynaptic internalization process. We showed that internalization produced by exogenous IT substance P was, however, not altered by IT gabapentin, indicating that the suppressant effects on evoked release was not due to a direct effect on the internalization process.

Facilitated pain states

The present work confirmed a potent antinociceptive effect of gabapentin given IT or IP upon phase 2, but not phase 1 of the formalin-evoked flinching, a finding consistent with those previously reported.¹⁻⁵ Consistent with a spinal action of gabapentin, the effective total dose was 200 μ g IT versus 60 mg IP (e.g., total dose for 200 mg/kg in a 300 g rat).

Correlation between effects on substance P release and antinociception

The functional relevance of the IT/IP gabapentin effects on release is suggested by the parallel between behaviorally relevant doses and those required to reduce release. We note, however, that after both IT and IP delivery, phase 2 flinching was reduced at lower doses than those required to produce reduction in NK1r internalization. Whether this represents a component of gabapentin's action on the flinching model which is not mediated by effects on peptidergic C-fibers, an effect upon other spinal systems or even nonspinal systems³⁴ is not known.

Effect of gabapentin on acute facilitation

One feature of gabapentin's actions is its effects upon facilitated pain states which may be initiated within minutes of stimulation (phase 2 flinching and tissue injury-evoked hyperalgesia) but not upon acute nociception (phase 1 flinching, thermal thresholds).^{1-5,35} An example of the acuity of this effect is the observation that while IT or IP gabapentin or pregabalin (S(+)-3-isobutyl-gamma aminobutyric acid) has no effect upon acute thermal escape latencies, they reverse the thermal hyperalgesia evoked in seconds to minutes by IT substance P, an effect reflecting a postsynaptic effect mediated by substance P on the NK1r. The specificity of this effect is emphasized by the observation that an isomer of pregabalin (R(-)-3-isobutyl-gamma aminobutyric acid) with lower $\alpha 2\delta 1$ binding affinity was less active

in vivo.³⁶ This differential effect upon acute versus facilitated states distinguishes the actions of gabapentin from those of other drugs which block small afferent release, such as opiates.³⁷ The observation that gabapentin did not block NK1r internalization evoked by IT substance P and yet blocked the hyperalgesia evoked by IT substance P, raises the interesting likelihood that gabapentin has several sites of spinal action, one of which is presynaptic on the afferent (to block release of substance P and likely other afferent transmitters such as glutamate) and one of which appears not to be on the primary afferent¹³. Where the nonafferent action is we do not know, but the lack of effect upon internalization evoked by IT substance P emphasizes that the gabapentin effect was not due to an artifact of an action on the internalization process.

Several mechanisms for this acute action on facilitated states present themselves. i) The effects upon presynaptic release initiated by persistent small afferent input, as generated during the first phase,³⁸ may represent distinct processes in the presynaptic terminal leading to release. Protein kinase C and adenylyl cyclase activation exert a potent facilitatory effect upon glutamate release in trigeminal slices and this facilitation (but not acute release) is blocked by gabapentin, perhaps through reduced phosphorylation of associated VSCCs.¹⁴ ii) $\alpha 2\delta 1$ subunits are distributed in a variety of DRG cells.¹⁸ Accordingly, there are likely effects upon terminals that are not peptidergic. iii) In dorsal horn, $\alpha 2\delta 1$ may be associated with nonafferent terminals. As noted, gabapentin blocks hyperalgesia initiated by spinal NK1 activation.³⁶ In this vein, gabapentin blocks substance P-evoked NF- κ B activation in cell systems by blockade of I κ B- α degradation, thereby preventing translocation of p65 to the nucleus.³⁹

Mechanisms of actions of gabapentins

As discussed above, the membrane level, the role of the binding $\alpha 2\delta 1$ site in directly regulating calcium channel function has been controversial. The present studies, however, do emphasize the presynaptic effect upon small afferent terminal-releasing properties. Other mechanisms which may be associated with the actions of gabapentin, include the trafficking of intracellular VSCC, prevented by gabapentin,¹⁸ and spouting after nerve injury. Thus, $\alpha 2\delta 1$ binds the thrombospondin family of extracellular matrix proteins which play a role in intracellular trafficking and synaptogenesis.^{40,41} Importantly these proteins are secreted by glia.⁴⁰ Previous work has indicated that even acute tissue injury or IT substance P can initiate glial activation as measured by, for example, phosphorylation of glial P38 mitogen-activated protein kinase.⁴²

As outlined above, however, an important element in the present study is that it draws attention to the acuity (seconds to minutes) of the biological effects of gabapentin including suppression of the release of at least one small afferent transmitter and the acute changes in facilitated spinal states. Thus, there is little doubt that upregulation of the $\alpha 2\delta 1$ subunits occur after nerve injury⁴³ and that chronic gabapentin exposure may lead to a down regulation of the associated VSCCs¹⁶ or other intracellular changes¹⁷ which alter VSCCs insertion into the cell membrane. The acute manifestations observed *in vivo* clearly represent an effect upon a constitutively expressed and functional spinal system that does not require additional changes in protein expression or channel mobilization or extended drug exposure.⁹ This profile of action thus forces us to consider the likelihood that additional mechanisms may be called into play which define the regulation exerted at the spinal level *in vivo* by the $\alpha 2\delta 1$ binding exhibited by gabapentin and its homologues.

In conclusion, the present work emphasizes that IT and systemic administration of gabapentin acutely regulate spinal release of substance P from small primary afferents at doses which are associated with its antihyperalgesic action. These results, along with previous electrophysiological¹³ and behavioral³⁶ work consistent with a postsynaptic

action, suggest that a number of mechanisms may reflect the acute actions of gabapentin and indirectly the role of the spinal $\alpha 2\delta 1$ subunit at which it binds.

Acknowledgments

We would like to thank Arbi Nazarian, Ph.D. for his assistance in setting up the internalization protocol.

Funding: National Institutes of Health: NIH-DA02110 Bethesda, MD (TLY).

References

1. Field MJ, Oles RJ, Lewis AS, McCleary S, Hughes J, Singh L. Gabapentin (neurontin) and S-(+)-3-isobutylgaba represent a novel class of selective antihyperalgesic agents. *Br J Pharmacol.* 1997; 121:1513–22. [PubMed: 9283683]
2. 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; 22:249–56. [PubMed: 9168217]
3. Yoon MH, Yaksh TL. Evaluation of interaction between gabapentin and ibuprofen on the formalin test in rats. *Anesthesiology.* 1999; 91:1006–13. [PubMed: 10519504]
4. Yoon MH, Yaksh TL. The effect of intrathecal gabapentin on pain behavior and hemodynamics on the formalin test in the rat. *Anesth Analg.* 1999; 89:434–9. [PubMed: 10439761]
5. Ortega-Varela LF, Herrera JE, Caram-Salas NL, Rocha-González HI, Granados-Soto V. Isobolographic analyses of the gabapentin-metamizol combination after local peripheral, intrathecal and oral administration in the rat. *Pharmacology.* 2007; 79:214–22. [PubMed: 17389816]
6. Stefani A, Spadoni F, Bernardi G. Gabapentin inhibits calcium currents in isolated rat brain neurons. *Neuropharmacology.* 1998; 37:83–91. [PubMed: 9680261]
7. Davies A, Hendrich J, Van Minh AT, Wratten J, Douglas L, Dolphin AC. Functional biology of the alpha (2) delta subunits of voltage-gated calcium channels. *Trends Pharmacol Sci.* 2007; 28:220–8. [PubMed: 17403543]
8. Field MJ, Cox PJ, Stott E, Melrose H, Offord J, Su TZ, Bramwell S, Corradini L, England S, Winks J, Kinloch RA, Hendrich J, Dolphin AC, Webb T, Williams D. Identification of the alpha2-delta-1 subunit of voltage-dependent calcium channels as a molecular target for pain mediating the analgesic actions of pregabalin. *Proc Natl Acad Sci U S A.* 2006; 103:17537–42. [PubMed: 17088553]
9. Luo ZD, Chaplan SR, Higuera ES, Sorkin LS, Stauderman KA, Williams ME, Yaksh TL. Upregulation of dorsal root ganglion (alpha) 2 (delta) calcium channel subunit and its correlation with allodynia in spinal nerve-injured rats. *J Neurosci.* 2001; 21:1868–75. [PubMed: 11245671]
10. Alden KJ, García J. Differential effect of gabapentin on neuronal and muscle calcium currents. *J Pharmacol Exp Ther.* 2001; 297:727–35. [PubMed: 11303064]
11. Sarantopoulos C, McCallum B, Kwok WM, Hogan Q. Gabapentin decreases membrane calcium currents in injured as well as in control mammalian primary afferent neurons. *Reg Anesth Pain Med.* 2002; 27:47–57. [PubMed: 11799505]
12. Li CY, Zhang XL, Matthews EA, Li KW, Kurwa A, Boroujerdi A, Gross J, Gold MS, Dickenson AH, Feng G, Luo ZD. Calcium channel alpha2delta1 subunit mediates spinal hyperexcitability in pain modulation. *Pain.* 2006; 125:20–34. [PubMed: 16764990]
13. Moore KA, Baba H, Woolf CJ. Gabapentin-- actions on adult superficial dorsal horn neurons. *Neuropharmacology.* 2002; 43:1077–81. [PubMed: 12504913]
14. Maneuf YP, Hughes J, McKnight AT. Gabapentin inhibits the substance P-facilitated K(+)-evoked release of [(3)H]glutamate from rat caudal trigeminal nucleus slices. *Pain.* 2001; 93:191–6. [PubMed: 11427331]
15. Fehrenbacher JC, Taylor CP, Vasko MR. Pregabalin and gabapentin reduce release of substance P and CGRP from rat spinal tissues only after inflammation or activation of protein kinase C. *Pain.* 2003; 105:133–41. [PubMed: 14499429]
16. Vega-Hernández A, Felix R. Down-regulation of N-type voltage-activated Ca²⁺ channels by gabapentin. *Cell Mol Neurobiol.* 2002; 22:185–90. [PubMed: 12363200]

17. Hebllich F, Tran Van Minh A, Hendrich J, Watschinger K, Dolphin AC. Time course and specificity of the pharmacological disruption of the trafficking of voltage-gated calcium channels by gabapentin. *Channels (Austin)*. 2008; 2:4–9. [PubMed: 18690052]
18. Bauer CS, Nieto-Rostro M, Rahman W, Tran-Van-Minh A, Ferron L, Douglas L, Kadurin I, Sri Ranjan Y, Fernandez-Alacid L, Millar NS, Dickenson AH, Lujan R, Dolphin AC. The increased trafficking of the calcium channel subunit $\alpha 2\delta$ -1 to presynaptic terminals in neuropathic pain is inhibited by the $\alpha 2\delta$ ligand pregabalin. *J Neurosci*. 2009; 29:4076–88. [PubMed: 19339603]
19. Mantyh PW, Allen CJ, Ghilardi JR, Rogers SD, Mantyh CR, Liu H, Basbaum AI, Vigna SR, Maggio JE. Rapid endocytosis of a G protein-coupled receptor: substance P evoked internalization of its receptor in the rat striatum in vivo. *Proc Natl Acad Sci U S A*. 1995; 92:2622–6. [PubMed: 7535928]
20. Willis WD Jr. The somatosensory system, with emphasis on structures important for pain. *Brain Res Rev*. 2007; 55:297–313. [PubMed: 17604109]
21. Kondo I, Marvizon JC, Song B, Salgado F, Codeluppi S, Hua XY, Yaksh TL. Inhibition by spinal μ - and δ -opioid agonists of afferent-evoked substance P release. *J Neurosci*. 2005; 25:3651–60. [PubMed: 15814796]
22. Yaksh TL, Rudy TA. Chronic catheterization of the spinal subarachnoid space. *Physiol Behav*. 1976; 17:1031–6. [PubMed: 14677603]
23. Malkmus SA, Yaksh TL. Intrathecal catheterization and drug delivery in the rat. *Methods Mol Med*. 2004; 99:109–21. [PubMed: 15131333]
24. Yaksh TL, Ozaki G, McCumber D, Rathbun M, Svensson C, Malkmus S, Yaksh MC. An automated flinch detecting system for use in the formalin nociceptive bioassay. *J Appl Physiol*. 2001; 90:2386–402. [PubMed: 11356806]
25. Abbadie C, Trafton J, Liu H, Mantyh PW, Basbaum AI. Inflammation increases the distribution of dorsal horn neurons that internalize the neurokinin-1 receptor in response to noxious and non-noxious stimulation. *J Neurosci*. 1997; 17:8049–60. [PubMed: 9315923]
26. Nazarian A, Christianson CA, Hua XY, Yaksh TL. Dexmedetomidine and ST-91 analgesia in the formalin model is mediated by $\alpha 2A$ -adrenoceptors: a mechanism of action distinct from morphine. *Br J Pharmacol*. 2008; 155:1117–26. [PubMed: 18846040]
27. Alexander SP, Mathie A, Peters JA. Guide to Receptors and Channels (GRAC), 3rd edition. *Br J Pharmacol*. 2008; 153:S1–209. [PubMed: 18347570]
28. Maneuf YP, Gonzalez MI, Sutton KS, Chung FZ, Pinnock RD, Lee K. Cellular and molecular action of the putative GABA-mimetic, gabapentin. *Cell Mol Life Sci*. 2003; 60:742–750. [PubMed: 12785720]
29. Boroujerdi A, Kim HK, Lyu YS, Kim DS, Figueroa KW, Chung JM, Luo ZD. Injury discharges regulate calcium channel $\alpha 2\delta 1$ subunit upregulation in the dorsal horn that contributes to initiation of neuropathic pain. *Pain*. 2009; 139:358–366. [PubMed: 18571852]
- 30.Coderre TJ, Kumar N, Lefebvre CD, Yu JS. Evidence that gabapentin reduces neuropathic pain by inhibiting the spinal release of glutamate. *J Neurochem*. 2005; 94:1131–9. [PubMed: 16092950]
31. Feng Y, Cui M, Willis WD. Gabapentin markedly reduces acetic acid-induced visceral nociception. *Anesthesiology*. 2003; 98:729–33. [PubMed: 12606919]
32. Takasusuki, T.; Xu, Q.; Yaksh, TL. Regulation of spinal substance P release by intrathecal calcium channel blockade and gabapentin. 13th World Congress on Pain; 2010; 10-A-1669
33. Marvizon JC, Wang X, Matsuka Y, Neubert JK, Spigelman I. Relationship between capsaicin-evoked substance P release and neurokinin 1 receptor internalization in the rat spinal cord. *Neuroscience*. 2003; 118:535–45. [PubMed: 12699788]
34. Tanabe M, Takasu K, Takeuchi Y, Ono H. Pain relief by gabapentin and pregabalin via supraspinal mechanisms after peripheral nerve injury. *J Neurosci Res*. 2008; 86:3258–64. [PubMed: 18655202]
35. Tuchman M, Barrett JA, Donevan S, Hedberg TG, Taylor CP. Central Sensitization and $\text{Ca(V)}\alpha 2\delta$ Ligands in Chronic Pain Syndromes: Pathologic Processes and Pharmacologic Effect. *J Pain*. May 14.2010

36. Partridge BJ, Chaplan SR, Sakamoto E, Yaksh TL. Characterization of the effects of gabapentin and 3-isobutyl-gamma-aminobutyric acid on substance P-induced thermal hyperalgesia. *Anesthesiology*. 1998; 88:196–205. [PubMed: 9447873]
37. Yaksh TL. Spinal systems and pain processing: development of novel analgesic drugs with mechanistically defined models. *Trends Pharmacol Sci*. 1999; 20:329–37. [PubMed: 10431212]
38. Puig S, Sorkin LS. Formalin-evoked activity in identified primary afferent fibers: systemic lidocaine suppresses phase-2 activity. *Pain*. 1996; 64:345–55. [PubMed: 8740613]
39. Park S, Ahn ES, Han DW, Lee JH, Min KT, Kim H, Hong YW. Pregabalin and gabapentin inhibit substance P-induced NF-kappaB activation in neuroblastoma and glioma cells. *J Cell Biochem*. 2008; 105:414–23. [PubMed: 18615578]
40. Eroglu C, Allen NJ, Susman MW, O'Rourke NA, Park CY, Ozkan E, Chakraborty C, Mulinyawe SB, Annis DS, Huberman AD, Green EM, Lawler J, Dolmetsch R, Garcia KC, Smith SJ, Luo ZD, Rosenthal A, Mosher DF, Barres BA. Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell*. 2009; 139:380–92. [PubMed: 19818485]
41. Kurshan PT, Oztan A, Schwarz TL. Presynaptic alpha2delta-3 is required for synaptic morphogenesis independent of its Ca²⁺-channel functions. *Nat Neurosci*. 2009; 12:1415–23. [PubMed: 19820706]
42. Svensson CI, Marsala M, Westerlund A, Calcutt NA, Campana WM, Freshwater JD, Catalano R, Feng Y, Protter AA, Scott B, Yaksh TL. Activation of p38 mitogen-activated protein kinase in spinal microglia is a critical link in inflammation-induced spinal pain processing. *J Neurochem*. 2003; 86:1534–44. [PubMed: 12950462]
43. Luo ZD, Calcutt NA, Higuera ES, Valder CR, Song YH, Svensson CI, Myers RR. Injury type-specific calcium channel alpha 2 delta-1 subunit up-regulation in rat neuropathic pain models correlates with antiallodynic effects of gabapentin. *J Pharmacol Exp Ther*. 2002; 303:1199–205. [PubMed: 12438544]

Figure 1.

Representative fluorescent microscopic images of neurokinin 1 receptor (NK1r) internalization at L6 lumbar spinal dorsal horn from rats administered intrathecal (IT) saline (A) and 200 μ g gabapentin (B). (A) An image of formalin-induced NK1r internalization. Note the lack of a homogeneous cell membrane and the presence of NK1 containing endosomes in the cytoplasm. (B) An image of NK1r in the lamina I. Notice the presence of a homogeneous cell membrane and the lack of NK1 containing endosomes in the cytoplasm. Images are taken at 60 $\times$. Scale bar is 10 μ m.

Figure 2.

Effects of intrathecal (IT) gabapentin on formalin-induced paw-flinching behavior and neurokinin 1 receptor (NK1r) internalization. (A) Time-effect curve of IT 100 and 200 µg gabapentin and saline on the formalin-induced paw-flinching. (B) IT 100 and 200 µg gabapentin significantly reduced the phase 2 of formalin-induced paw-flinching. (C) IT 200 µg, but not 100 µg gabapentin significantly reduced the formalin-induced NK1r internalization in the spinal segments L5-6 compared with saline. Data are presented as mean number of paw flinching and percentage of NK1r internalization with vertical bars showing S.E.M. *Represents a significant difference between saline-treated and drug-treated animals, $P < 0.05$. In panel 2A and 2B, $n=4-5$ rats per group; in panel 2C, $n=3-4$ rats per group.

Figure 3.

Effects of intraperitoneally (IP) gabapentin on formalin-induced paw-flinching behavior and neurokinin 1 receptor (NK1r) internalization. (A) Time-effect curve of IP 100 and 200mg/kg gabapentin and saline on the formalin-induced paw-flinching. (B) IP 100 and 200mg/kg gabapentin significantly reduced the phase 2 of formalin-induced paw-flinching. (C) IP 200mg/kg, but not 100mg/kg gabapentin significantly reduced the formalin-induced NK1r internalization in the spinal segments L5-6 compared with saline. Data are presented as mean number of paw flinching and percentage of NK1r internalization with vertical bars showing S.E.M. *Represents a significant difference between saline-treated and drug-treated animal, $P < 0.05$. In panel 3A and 3B, $n=4-6$ rats per group; in panel 3C, $n=3-4$ rats per group.

Figure 4.

The effects of gabapentin on the neurokinin 1 receptor (NK1r) internalization induced by exogenous substance P. (A) Intrathecal (IT) substance P (30nmol) produced a robust bilateral NK1r internalization at L4-6 levels of lumbar lamina I compared with saline. (B) Pretreatment of IT gabapentin (200μg) did not block NK1r internalization induced by IT substance P. (C, D) Representative fluorescent microscopic images of NK1r internalization in lamina I after substance P alone (C), substance P after gabapentin (D). Data are presented as mean number of percentage of NK1r internalization with vertical bars showing S.E.M. *Represents a significant difference in NK1r internalization between saline-treated and drug-treated animals, $P < 0.05$. Images are taken at 60 $\times$. Scale bar is 10μm. $n=3$ rats per group.

Table 1
Pharmacological disruption of calcium channel trafficking by the α 2delta ligand gabapentin
 Behavioral and motor effects of intrathecal (IT) and intraperitoneal (IP) gabapentin

Drug	Route	Dose	n	% showing side effect	Side effects
Gabapentin	IT	100 μ g	4	0	
		200 μ g	4	0	
Gabapentin	IP	100mg/kg	5	40	
		200mg/kg	4	50	Reduced spontaneous activity (> 120 min)