Review Article Open Access

Keywords: CB1/CB2 receptor; Hypersensitivity; Chemotherapy; Neuropathy; Intraperitoneally; Allodynia

Introduction

A common side-e ect of several di erent kinds of chemotherapy drugs is painful peripheral neuropathy. Examples of these groups of drugs include vinca alkaloids (like vincristine), compounds derived from taxanes (like paclitaxel), and compounds derived from platinum (like cisplatin). According to [1-4], and other studies, the incidence and severity of chemotherapy-induced neuropathy are in uenced by the type of cancer, the dose schedule, the choice of chemotherapeutic drug, and the existence of concurrent medical issues. It has been

nociception is suppressed by the mixed CB1/CB2 receptor agonist WIN55, 212-2 via a CB1 mechanism [26]. Nevertheless, little is known about the mechanisms behind the emergence of excruciating peripheral neuropathies brought on by various chemotherapy drugs (for a review, see [27]. Di erent symptoms of neuropathic pain complied with the International Association for the Study of Pain's recommendations for treating animals [28]. Following the relevant institutional procedures, bedding containing metabolized vincristine was handled as bio hazardous waste and disposed of.

that was suspended 9 cm above a level platform. In animals treated with vincristine and given either a vehicle (n ¼ 6) or WIN55, 212-2 (2.5 mg kg—1 i.p.; n ¼ 6), catalepsy was reassessed. A di erent set of mice treated with vincristine (who did not have thermal test) were given AM1241, which is 2.5 mg kg-1 i.p.; n ¼ 6. For example, WIN55, 212-2 (2.5 or 10 mg kg—1 i.p.; n ¼ 6 per group) was given to two groups of otherwise naive rats. e time I stood there at the bar was measured for each group in triplicate at 30, 45, and 60 minutes a er the medication injection.

Examinations of statistics

For repeated measures, analysis of variance (ANOVA) or planned comparison unpaired t-tests were used for data analysis when applicable. e Greenhouse-Geissner adjustment was implemented for every element that was repeated. Additionally, post-drug thresholds and pre-vincristine thresholds were compared using paired t-tests. (baseline) cuto points. Using the following formula, the percent (%) reversal of mechanical allodynia was determined at the moment of maximal cannabis anti-allodynic e cacy: Using Fisher's protected least signi cant di erence (PLSD) test, post hoc comparisons were executed. It was decided that Po0.05 was statistically signi cant.

Chemicals and drugs

Tocris Cookson provided the vincristine sulphate (Ellisville, MO, USA). R(þ)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl] WIN55,212-23-de pyrrolo[1,2,3]One (1) 1,4-benzoxazin-ylWIN55, 212-3 (S(-)-[2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]),-(1-naphthalenyl)methanone mesylate3-de pyrrolo[1,2,3]One (1) 4,4-benzoxazinylSigma Aldrich (St. Louis, MO, USA) provided the morphine sulfate, b-cyclodex-trin, and-(1-naphthalenyl)methanone mesylate salt. (S, R)AM1241, ((R,S)-(2-iodo-5-nitro-phenyl)-[l-(l-methyl-piperidin-2-ylmethyl)-lH-indol-3-yl]-methanone) was produced. sized at one of the authors' laboratories (AM). Asymmetric

Figure 2: (a) Vincristine did not induce hypersensitivity to thermal stimulation relative to the control condition. (b) The same vincristine-treated animals showed robust mechanical allodynia (on day 12). Data are means7s.e.m. **P<0.001 diﬀerent from control conditions (ANOVA). N ¼ 6-12 per group.

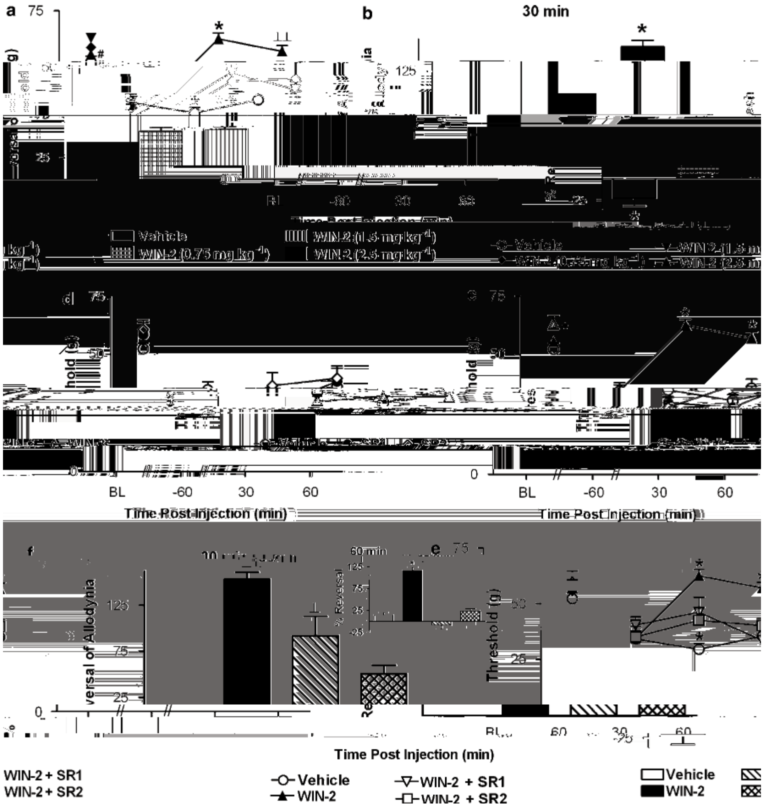

Figure 3: (a) The CB1/CB2 agonist WIN55, 212-2 (WIN-2; 2.5, 1.5 and 0.75 mg kg—1 i.p.) induced a dose-dependent suppression of vincristine-induced mechanical allodynia, as demonstrated by an increase in the mechanical paw withdrawal threshold (on day 12). In all panels, BL denotes the baseline, day 0, paw withdrawal threshold assessed before vincristine or saline treatment. (b) WIN55, 212-2 (2.5 mg kg—1 i.p.) produced a maximal reversal of mechanical allodynia at 30 min post-injection. (c) WIN55, 212-2 (2.5 mg kg—1 i.p.) suppressed vincristine-evoked mechanical allodynia relative to the receptor-inactive enantiomer WIN55, 212-3 (WIN-3; 2.5 mg kg—1 i.p.) or vehicle. (d) The CB1 antagonist SR141716 (SR1; 2.5 mg kg—1 i.p.) and the CB2 antagonist SR144528 (SR2; 2.5 mg kg—1 i.p.) did not alter vincristine-induced mechanical allodynia relative to vehicle. (e) Blockade of WIN55, 212-2-induced anti-allodynia by SR141716 and SR144528.

mechanical allodynia was reversed by the intermediate and low doses of WIN55, 212-2 (0.75 and 1.5 mg kg—1 i.p.) (Po.01 for all comparisons).

e large amount 30 minutes a er injection, the highest suppression of mechanical hypersensitivity was achieved with a dose of WIN55, 212-2 (2.5 mg kg—1 i.p.) (Po0.002 for all comparisons; Figure 3b). According to Figure 3c, WIN55, 212-2 (2.5 mg kg—1 i.p.) suppressed mechanical hypersensitivity in comparison to treatment with vehicle or the receptor-inactive enantiomer WIN55, 212-3 (2.5 mg kg—1 i.p.); this increase in mechanical withdrawal thresholds was caused by WIN55, 212-2 and was receptor-mediated (F2, 21 17.78, Po0.0002 for each comparison). Paw withdrawal thresholds were likewise raised in comparison to day 12 reinjection thresholds by the active enantiomer but not by the inactive one (F4, 42 ¼ 11.236, Po0.0005; Figure 3c). At no stage did the mechanical withdrawal thresholds of the animals treated with WIN55, 212-3 change from the vehicle.

Pharmacological specificity

In vincristine-treated rats, administration of the CB1-selective antagonist SR141716 (2.5 mg kg-1 i.p.) or the CB2-selective antagonist SR144528 (2.5 mg kg—1 i.p.) did not alter paw withdrawal thresholds relative to vehicle (Figure 3d).However, both antagonists blocked the suppression of vincristine-evoked mechanical allodynia induced by WIN55,212-2 (F3,28¼5.79, Po0.004; Po0.05 for each comparison; Figure 3e) and this blockade was time-dependent(F6,56 ¼ 9.51, Po0.0002). Post hoc comparisons failed to reveal a di erential blockade of the anti-allodynic e ects of WIN55, 212-2 following treatment with either antagonist. Paw withdrawal thresholds were higher in groups receiving WIN55, 212-2 alone compared to either antagonist coadminis-tration group. Partial and complete blockade of the WIN55,212-2-induced attenuation of vincristine-induced mechanical hypersensitivity was observed at 30 and 60 min post-injection, respectively (Po0.05 for each comparison; Figure 3e).WIN55,212-2 (2.5 mg/kg i.p.) produced 4100% reversal of vincristine-evoked mechanical allodynia relative to vehicle treatment at 30 min post-injection ($F_{3,28}$ 4.009, Po0.02; Figure 3f). At this time point, SR144528 (Po0.005, planned comparison t-test), but not SR141716, reliably attenuated the anti-allodynic e ects of WIN55, 212-2. Planned comparisons failed to reveal signi cant di erences in reversal of vincristine-evoked mechanical allodynia observed following WIN55,212-2 coadministration with either SR144528 or SR141716 (P40.26). By 60 min post-injection, both SR141716 and SR144528 produced a complete reversal of the WIN55,212-2-induced suppression of mechanical allo-dynia ($F_{3,28}$ ¼ 9.123, Po0.0003; Po.002 for all comparisons; Figure 3f, inset). Assessment of mechanical allodynia following systemic administration of AM1241 and morphine WIN55, 212-2 (2.5 mg kg—1 i.p.) and morphine (8 mg kg—1 i.p.) suppressed vincristine-evoked mechanical allodynia ($F_{4,31}$ ¼ 9.513, Po0.0002; Figure 4a) relative to treatment with either vehicle, the CB2-selective agonist AM1241 or the lower dose (2.5 mg kg—1 i.p.) of morphine (Po0.01 for each comparison). e time course of anti-allodynic e ects observed was di erentially a ected by the experimental treatments ($F_{8,62}$ ¼ 3.926, Po0.002). e suppression of vincristine-evoked mechanical allodynia induced by WIN55, 212-2 (2.5 mg kg—1 i.p.) was comparable to the high dose (8 mg kg—1 i.p.) of morphine. By contrast, paw with-drawal thresholds in groups receiving the lower dose of morphine (2.5 mg kg—1 i.p.) did not di er from vehicle at any time point. A le ward shi in the dose-response curve for post-drug paw withdrawal thresholds was also observed for WIN55, 212-2 relative to morphine (Figure 4b). AM1241 (2.5 mg kg—1 i.p.) also suppressed vincristine-evoked mecha-nical allodynia relative to vehicle and the low dose of morphine (2.5 mg kg—1 i.p.). is suppression was maximal at 30 min post-injection (Po0.05 for all comparisons; Figure 4a). e anti allodynic e ect of WIN55,212-2 (2.5 mg kg—1 i.p.) was greater (Po0.05) and of longer duration than that induced by AM1241 (Figure 4a). e AM1241-induced suppression of vincristine-induced mecha-nical hypersensitivity was similar to that induced by the low and middle doses of WIN55,212-2 (0.75 and 1.5 mg kg—1 i.p., respectively); thresholds were elevated at 30 min post-injection and returned to vehicle levels by 60 min post-drug (Po0.04 for all comparisons; Figures 4b and c). e AM1241-induced suppression of mechanical allodynia was mediated by CB2 receptors ($F_{2,21}$ ¼ 8.58, Po0.002, Figure 4d). e anti-allodynic e ects of AM1241 were blocked by the CB2 antagonist SR144528 (2.5 mg kg—1 i.p.; Po0.003) but not by the CB1 antagonist SR141716.

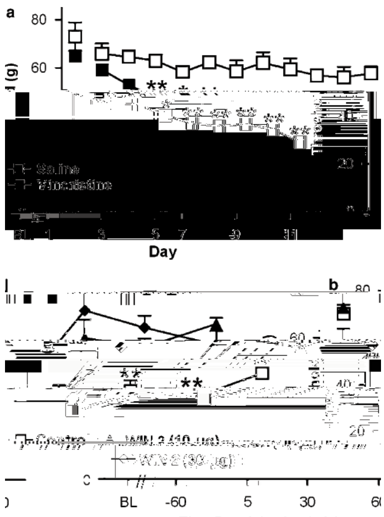

Figure 4 (a) Time course of development of vincristine-induced mechanical allodynia in rats implanted with i.t. catheters. (b) The CB1/CB2 agonist WIN55, 212-2 (WIN-2; 10 and 30 mg i.t.) suppressed vincristine-induced mechanical allodynia. (c) WIN55, 212-2 (10 mg i.t.) suppressed vincristine-evoked mechanical allodynia relative to the receptor-inactive enantiomer WIN55, 212-3 (WIN-3; 10 mg i.t.) or the control condition. Data are means7s.e.m. **P<0.01, *P<0.05 diferent from all groups, ##Po0.01 diferent from WIN55, 212-2 (10 mg i.t.) (ANOVA and Fisher's PLSD post hoc test). N ¼ 6-9 per group.

Assessment of spinal site of cannabinoid action

Mechanical withdrawal thresholds did not di er between vincristine-treated groups receiving the b-cyclodextrin vehicle (i.t.) and controls that were surgically implanted with catheters but did not receive an injection (i.t.). erefore, these groups were pooled into a single control group for subsequent statistical analysis of drug e ects. In vincristine-treated rats, administration of the CB1/ CB2 agonist WIN55, 212-2 (10 and 30 mg i.t.) increased mechanical withdrawal thresholds relative to either the control condition ($F_{2,19}$ ¼ 11.499, Po0.0006, Figure 5b) or to day 12 preinjection levels ($F_{6,57}$ ¼ 2.698, Po0.04; Figure 5b). Post hoc analyses failed to discriminate between the two doses of WIN55, 212-2 (10 and 30 mg i.t.) at any

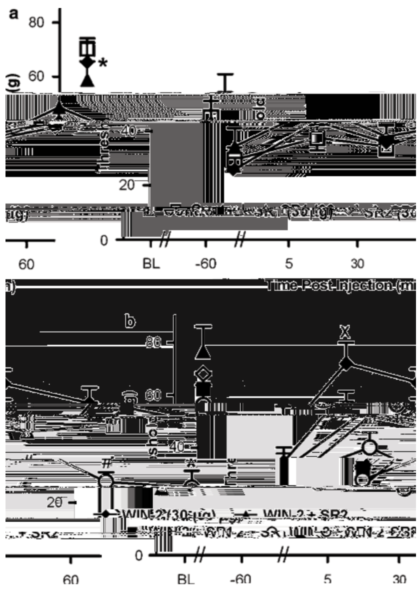

Figure 5: (a) The CB1 antagonist SR141716 (SR1; 30 mg i.t.) and the CB2 antagonist SR144528 (SR2; 30 mg i.t.) did not alter vincristine-induced mechanical allodynia relative to vehicle. (b) WIN55, 212-2 (WIN-2; 30 mg i.t.) increased mechanical withdrawal thresholds relative to all other groups. Concurrent (i.t.) administration of SR141716 and SR144528 blocked the WIN55, 212-2-induced sup-pression of vincristine-evoked mechanical allodynia. Data are mean 7s.e.m. *P<0.05 diferent from all groups, #Po0.05 diferent from WIN55, 212-2 þ SR2 and WIN55, 212-2 (30 mg i.t.) XPo0.05 diferent from WIN55, 212-2 þ SR2 and WIN55,

time point. e WIN55, 212-2-induced increase in mechanical withdrawal thresholds was receptor-mediated (F2, 19 7.152, Po0.005; Figure 5c). WIN55, 212-2 (10 mg i.t.) suppressed vincristine-evoked mechanical hypersensitivity relative to treatment with its receptor-inactive enantiomer WIN55, 212-3 (10 mg, i.t) or the control condition (Po0.02 for each comparison). Mechanical withdrawal thresholds in WIN55,212-3-treated animals did not di er from control levels at any time point (Figure 5c).Spinal administration of either SR141716 (30 mg i.t.) or SR144528 (30 mg i.t.) did not alter paw withdrawal thresholds relative to the control condition (Figure 6a). However, coadministration (i.t.) of both SR141716 and SR144528 concurrently with WIN55, 212-2 blocked the cannabinoid induced suppression of vincristine-evoked mechanical allodynia (F4, 33 ¼ 4.503, Po0.006, Po0.05 for each comparison; Figure 5b). By contrast, a trend toward partial blockade of WIN55, 212-2-induced anti-allodynia was observed following i.t. administration of the agonist with either the CB1 (Po0.13) or CB2 (Po0.08) antagonist alone, respectively. Planned comparisons con rmed that the CB2 antagonist induced a partial blockade of the anti allodynic e ects of WIN55, 212-2 at 5 and 30 min post-injection (Po0.05 for each comparison). Intrathecal co-administration of both antagonists with WIN55, 212-2 blocked the cannabinoid-induced suppression of vincristine-evoked mechanical hypersensitivity at all-time points (Po0.006 for each comparison; Figure 5b).

Assessment of peripheral site of cannabinoid action

e i.pl. Injection lowered mechanical withdrawal thresholds relative to day 12 preinjection levels (F1, 22 7.47; Po0.02) (Figure 6), consistent with the development of hypersensitivity at the site of injection. Enhanced hypersensitivity was di erentially observed in the injected paw.

Assessment of spinal site of cannabinoid action

Mechanical withdrawal thresholds did not di er between vincristine-treated groups receiving the b-cyclodextrin vehicle (i.t.) and controls that were surgically implanted with catheters but did not receive an injection (i.t.). erefore, these groups were pooled into a single control group for subsequent statistical analysis of drug e ects. In vincristine-treated rats, administration of the CB1/CB2 agonist WIN55, 212-2 (10 and 30 mg i.t.) increased mechanical withdrawal thresholds relative to either the control condition (F2, 19 ¼ 11.499, Po0.0006, Figure 4b) or to day 12 preinjection levels (F6, 57 ¼ 2.698, Po0.04; Figure 4b). Post hoc levels that were lower than baseline in

[39] and traumatic nerve injury [40], the same local dose used here (30 mg i.pl.) reduced mechanical allodynia; however, in our study, it was unable to reduce vincristine-induced neuropathy or attenuate paclitaxel neuropathy [41]. e paw withdrawal thresholds in the non-injected paw were likewise raised above baseline (previncristine) levels by local injection of WIN55, 212-2 (30 mg i.pl.), however this did not alleviate the hypersensitivity that was noted at the injection site. Paw withdrawal threshold variations in the non-injected paw may be related to cannabis leakage into the systemic circulation. WIN55,212-2 with a larger local dose of 150 mg i.pl., which causes de nite systemic e ects [40] removed the hypersensitivity at the site of the injection of IPL. Nevertheless, this dosage did not normalize paw withdrawal thresholds to pre vincristine levels and did not decrease vincristine-evoked mechanical allodynia in comparison to pre injection levels. Central sensitization is brought on by vincristine in wide dynamic range neurons in the spinal cord, such as aberrant spontaneous activity, wind-up, and a er-discharge reactions to mechanical stimulation applied above threshold [42]. e reported neuropathy brought on by chemotherapy may be mediated by these abnormal neurophysiological reactions. Cannabinoids inhibit spinal wide dynamic range neurons and C- bre-mediated responses by means of CB1 [43,44] or CB2 [45] speci c mechanisms. To understand the neurophysiological underpinnings of cannabinoid-mediated reduction of chemotherapy-induced neuropathy, more research is necessary [46]. Presynaptic facilitation, or enhanced primary a erent glutamate release, could potentially be involved in the aberrant behavioral phenotype and central sensitization brought on by chemotherapy. Reduced protein levels for the excitatory amino acid synthase (EASN), glial glutamate transporter-1 (GLT-1), and glutamate-aspartate trans-porter (GLAST) are consistent with this theory. A er paclitaxel treatment, carrier-1 (EAAC1) is seen [47]. Notably, however, glutamate and NMDA receptor antagonists do not restore hyperalgesia in models of chemotherapy-induced neuropathy [48], but they do in a nerve-injury model [49]. erefore, di erent pathways could be involved in the development of neuropathic nociception brought on by chemotherapy and traumatic nerve injury, respectively. An increase in intracellular Ca2 + [50] may be brought about by abnormal primary a erent input, presynaptic and/or descending [51,52] facilitation, and chemotherapy-induced dysregulation of calcium homoeostasis [53]. A T-type calcium antagonist called ethosuximide, along with other medications that lower intra-and extracellular Ca2 þ, also lower mechanical hypersensitivity brought on by vincristine [48,53]. Further research is necessary to ascertain whether the cannabis suppression of chemotherapy-induced neuropathy is connected to the cannabinoid suppression of central sensitization and Ca2 conductance [54,55].

Conclusion

Our ndings directly demonstrate the involvement of spinal sites of action in the inhibition of chemotherapy-induced neuropathy mediated by CB1 and CB2 receptors. Remarkably, rats with traumatic nerve injury in their spinal cords have higher levels of CB2 receptor mRNA and protein. A functional involvement for spinal CB2 receptors in neuropathic pain states is suggested by the direct spinal injection of a CB2 agonist, which also reduces mechanically evoked responses in wide dynamic range neurons in neuropathic rats but not in sham-operated rats.

References

1.

24. Zhang J, Hofert C, Vu HK, Groblewski T, Ahmad S, et al. (2003) Induction of CB2 receptor expression in the rat spinal cord of neuropathic but not infammatory chronic pain models. Eur J Neurosci. 17: 2750-2754.

25. Wotherspoon G, Fox A, McIntyre P, Colley S, Bevan S, Winter J (2005) Peripheral nerve injury induces cannabinoid receptor 2 protein expression in rat sensory neurons. Neuroscience. 135: 235-245.

26. Pascual D, Goicoechea C, Suardiaz M, Martin MI (2005) A cannabinoid agonist, WIN 55,212-2, reduces neuropathic nocicep-tion induced by paclitaxel in rats. Pain. 118: 23-34.

27. Cata JP, Weng HR, Lee BN, Reuben JM, Dougherty PM (2006b) Clinical and experimental fndings in humans and animals with chemotherapy-induced peripheral neuropathy. Minerva Anesthesia. 72: 151-169.

28. Zimmermann M (1983) Ethical guidelines for investigations of experimental pain in conscious animals. Pain. 16: 109-110.

29. Weng HR, Cordella JV, Dougherty PM (2003) Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia. Pain. 103: 131-138.

30. Ibrahim MM, Rude ML, Stagg NJ, Mata HP, Lai J, et al. (2006) CB2 cannabinoid receptor mediation of antinociception. Pain. 122: 36-42.

31. LaBuda CJ, Little PJ (2005) Pharmacological evaluation of the selective spinal nerve ligation model of neuropathic pain in the rat. J Neurosci Methods. 144: 175-181.

32. Joshi SK, Hernandez G, Mikusa JP, Zhu CZ, Zhong C, et al. (2006) Comparison of ant nociceptive actions of standard analgesics in attenuating capsaicin and nerve-injury-induced mechanical hypersensitivity. Neuroscience 143: 587-596.

33. Ibrahim MM, Deng H, Zvonok A, Cockayne DA, Kwan J, et al. (2003) Activation of CB2 cannabinoid receptors by AM1241 inhibits experimental neuropathic pain: pain inhibition by receptors not present in the CNS. Proc Natl Acad Sci. 100: 10529-10533.

34. Yaksh TL, Rudy TA (1976) Chronic catheterization of the spinal subarachnoid space. Physiol Behav. 17: 1031-1036.

35. Hohmann AG, Herkenham M (1998a) Regulation of cannabinoid and mu opioid receptor binding sites following neonatal capsaicin treatment. Neurosci Lett. 252: 13-16.

36. Pertwee RG, Ross TM (1991) Drugs which stimulate or facilitate central cholinergic transmission interact synergistically with delta-9-tetrahydrocannabinol to produce marked catalepsy in mice. Neuropharmacology. 30: 67-71.

37. Martin WJ, Hohmann AG, Walker JM (1996) Suppression of noxious stimulus-evoked activity in the ventral posterolateral nucleus of the thalamus by a cannabinoid agonist: correlation between electrophysiological and antinociceptive efects. J Neurosci. 16: 6601-6611.

38. Hohmann AG, Tsou K, Walker JM (1998b) Cannabinoid modulation of wide dynamic range neurons in the lumbar dorsal horn of the rat by spinally administered WIN55, 212-2. Neurosci Lett. 257: 119-122.

39. Ulugol A, Karadag HC, Ipci Y, Tamer M, Dokmeci I (2004) The efect of WIN 55,212-2, a cannabinoid agonist, on tactile allodynia in diabetic rats. Neurosci let. 371: 167-170.

40. Fox A, Kesingland A, Gentry C, McNair K, Patel S, et al. (2001) The role of central and peripheral Cannabinoid1 receptors in the antihyperalgesic activity of cannabinoids in a model of neuropathic pain. Pain. 92: 91-100.

41. Pascual D, Goicoechea C, Suardiaz M, Martin MI (2005) A cannabinoid agonist, WIN 55,212-2, reduces neuropathic nociception induced by paclitaxel in rats. Pain. 118: 23-34.

42. Weng HR, Cordella JV, Dougherty PM (2003) Changes in sensory processing in the spinal dorsal horn accompany vincristine-induced hyperalgesia and allodynia. Pain. 103: 131-138.

43. Strangman NM, Walker JM (1999) The cannabinoid WIN 55,212-2 inhibits the activity-dependent facilitation of spinal nociceptive responses. J Neurophys. 81: 472-477.

44. Drew LJ, Harris J, Millns PJ, Kendall DA, Chapman V (2000)

45. Nackley AG, Zvonok AM, Makriyannis A, Hohmann AG (2004) Activation of cannabinoid CB2 receptors suppresses C-fber responses and windup in spinal wide dynamic range neurons in the absence and presence of infammation. J Neurophysiol 92: 3562-3574.

46. Hohmann AG (2005) A cannabinoid pharmacotherapy for chemo-therapy-evoked painful peripheral neuropathy. Pain. 118: 3-5.

47. Cata JP, Weng HR, Chen JH, Dougherty PM (2006a) Altered discharges of spinal wide dynamic range neurons and down-regulation of glutamate transporter expression in rats with paclitaxel-induced hyperalgesia. Neuroscience. 138: 329-338.

48. Flatters SJ, Bennett GJ (2004) Ethosuximide reverses paclitaxel and vincristine-induced painful peripheral neuropathy. Pain. 109: 150-161.

49. Mao J, Price DD, Mayer DJ (1995) Experimental mononeuropathy reduces the antinociceptive efects of morphine: implications for common intracellular mechanisms involved in morphine tolerance and neuropathic pain. Pain. 61: 353-364.

50. Kawamata M, Omote K (1996) Involvement of increased excitatory amino acids and intracellular Ca2 þ concentration in the spinal dorsal horn in an animal model of neuropathic pain. Pain. 68: 85-96.

51. Porreca F, Burgess SE, Gardell LR, Vanderah TW, Malan Jr TP, et al. (2001) Inhibition of neuropathic pain by selective ablation of brainstem medullary cells expressing the mu-opioid receptor