Pregabalin Suppresses Spinal Neuronal Hyperexcitability and Visceral Hypersensitivity in the Absence of Peripheral Pathophysiology

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ABSTRACT

Background: Opioid-induced hyperalgesia is recognized in the laboratory and the clinic, generating central hyperexcitability in the absence of peripheral pathology. We investigated pregabalin, indicated for neuropathic pain, and ondansetron, a drug that disrupts descending serotonergic processing in the central nervous system, on spinal neuronal hyperexcitability and visceral hypersensitivity in a rat model of opioid-induced hyperalgesia.

Methods: Male Sprague-Dawley rats (180–200 g) were implanted with osmotic mini-pumps filled with morphine ($90\ \mu g \cdot \mu l^{-1} \cdot h^{-1}$) or saline (0.9% w/v). On days 7–10 in isoflurane anesthetized animals, we evaluated the effects of (1) systemic pregabalin on spinal neuronal and visceromotor responses, and (2) spinal ondansetron on dorsal horn neuronal response. Messenger ribonucleic acid concentrations of $\alpha_2\delta$-1, 5HT3A, and μ-opioid receptor in the dorsal root ganglia of all animals were analyzed.

Results: In morphine-treated animals, evoked spinal neuronal responses were enhanced to a subset of thermal and mechanical stimuli. This activity was attenuated by pregabalin (by at least 71%) and ondansetron (37%); the visceromotor response to a subset of colorectal distension pressures was attenuated by pregabalin (52.8%; n = 8 for all measures, $P < 0.05$). Messenger ribonucleic acid concentrations were unchanged.

Conclusions: The inhibitory action of pregabalin in opioid-induced hyperalgesia animals is neither neuropathy-dependent nor reliant on up-regulation of the $\alpha_2\delta$-1 subunit of voltage-gated calcium channels—mechanisms proposed as being essential for pregabalin's efficacy in neuropathy. In opioid-induced hyperalgesia, which extends to colonic distension, a serotonergic facilitatory system may be up-regulated, creating an environment that is permissive for pregabalin-mediated analgesia without peripheral pathology.

What We Already Know about This Topic

- Chronic opioid therapy results in hypersensitivity in animals to peripheral stimuli, and this phenomenon depends on activation of serotonin receptors of the 5HT3 subtype
- Whether opioid-induced hypersensitivity extends to visceral stimuli is not known

What This Article Tells Us That Is New

- In anesthetized rats, 1 week of exposure to morphine infusion resulted in hypersensitivity to colonic distention and enhanced spinal cord neuronal response to peripheral stimulation
- Enhanced responses were blocked by the neuropathic pain analgesic pregabalin and by the 5HT3 antagonist ondansetron

THE mode of action of drugs used in the clinic may allow their effectiveness to extend to more than one type of pain. Pain often involves disordered function within peripheral processes, but these processes may not be necessary for central actions of drugs.

Opiate analgesics remain the primary source of pain relief in conditions ranging from acute and postoperative pain to chronic pain. Unfortunately, chronic opioid consumption

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Received from the Department of Neuroscience, Pharmacology, and Physiology, University College London, London, United Kingdom. Submitted for publication September 5, 2010. Accepted for publication February 18, 2011. Supported by the University College London Grant no. FENDV, National Institutes of Health (Bethesda, Maryland) Grant no. FEPB (to Dr. Porreca), GlaxoSmithKline (Harlow, United Kingdom) Biotechnology and Biological Sciences Research Council Collaborative Awards in Science and Engineering Grant no. FEF2, and Medical Research Council (London, United Kingdom) Grant no. G0700368. Presented in part at the 6th Triennial Congress of the European Federation of International Association for the Study of Pain Chapters, Lisbon, Portugal, September 9, 2009.

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can generate opioid-induced hyperalgesia (OIH), which is characterized by a lowered pain threshold, and is recognized in the clinic and laboratory.[1–4] Mechanisms proposed to be essential for the development of OIH include neuroadaptive alterations in the pain modulatory circuitry[5–7] and enhanced descending facilitation to the spinal cord from higher central nervous system (CNS) centers, which may lead to spinal cord hyperexcitability that supports the development and maintenance of OIH.[8] However, evidence for nociceptive sensitivity to visceral stimuli in OIH animal models is lacking.

Although pregabalin attenuates enhanced neuronal responses to peripheral somatic inputs after nerve injury, it also shows analgesic efficacy in acute colorectal distension (CRD) models of visceral pain.[9–11] Analgesic actions of pregabalin in neuropathy are proposed to be state-dependent and rely on up-regulation of the $\alpha_2\delta$-1subunit of voltage-gated calcium channels to which it binds to disrupt channel trafficking.[12,13] Pregabalin antinociception is also thought to involve interactions with a spinal-bulbo-spinal loop that comprises projection neurons in the superficial dorsal horn and brainstem descending 5-HT3 receptor–mediated facilitations.[14] Gabapentinoids may activate noradrenergic neurons in the brainstem *via* a glutamate-dependent mechanism, producing antihypersensitivity after nerve injury.[15] They have previously been shown to prevent behavioral OIH.[16]

In a rat model of OIH, we confirmed the presence of spinal neuronal hyperexcitability in the absence of peripheral pathology. In addition, we investigated whether visceral hypersensitivity was present. We examined the efficacy of systemic pregabalin on reducing hypersensitivities and clarified whether any inhibitory actions observed were attributable to up-regulation of $\alpha_2\delta$-1 subunits. We also investigated whether spinal ondansetron, a selective 5HT3-receptor antagonist, attenuated dorsal horn neuronal hypersensitivity in a similar fashion to that which occurs in a spinal nerve injury model of neuropathy—and whether it interacted with the analgesic actions of pregabalin.

Materials and Methods

Animal experiments, approved by the United Kingdom Home Office (London), were performed according to guidelines set by personal and project licenses complying with the United Kingdom Animals (Scientific Procedures) Act 1986 and University College London biologic services ethical review committee.

Sustained Morphine Administration

Male Sprague-Dawley rats (180–200 g) were implanted subcutaneously with osmotic mini-pumps (ALZET Osmotic Pumps, Cupertino, CA) filled with saline (0.9%, 0.5 μl/h) or morphine (90 $\mu g \cdot \mu l^{-1} \cdot h^{-1}$) under isoflurane anesthesia (1.5% v/v) delivered in a gaseous mix of N_2O (66%) and oxygen (33%). Investigators were blinded as to group assignments.

Behavioral Tests

Behavioral responses were recorded on days 5–8. Plantar hind paw sensitivity to mechanical punctate stimulation was assessed through measurement of hind paw withdrawal frequency to a trial of 10 applications (5 s each) of calibrated von Frey filaments (increasing bending force 1, 6, and 8 g). Cold sensitivity was assessed as hind paw withdrawal frequency to five applications of acetone.

Electrophysiology

In vivo electrophysiology experiments were conducted on days 7–10 in anesthetized rats, as previously described.[17] In brief, animals were anesthetized and maintained for the experiment with isoflurane (1.5% v/v) delivered in a gaseous mix of N_2O (66%) and oxygen (33%). A laminectomy exposed L4-L5 segments of the spinal cord. Extracellular recordings were made from deep dorsal horn wide dynamic range (WDR) spinal neurons (lamina V-VI) using parylene-coated tungsten electrodes (A-M Systems, Sequim, WA).

Electrical activation of WDR neurons was delivered *via* stimulating needles inserted into the peripheral receptive field. A train of 16 transcutaneous electrical stimuli was applied at three times the threshold current for C-fiber activation of the spinal neuron. A poststimulus histogram was constructed and responses evoked by Aβ- (0–20 ms), Aδ- (20–90 ms), and C-fibers (90–350 ms), or postdischarge (350–800 ms), were separated and quantified on the basis of latency. Input, a measure of presynaptic activity and wind-up, a measure of spinal postsynaptic hyperexcitability was quantified.[17] Natural stimulation of the WDR neurons was delivered *via* mechanical punctate (2, 8, 26, and 60 g) and thermal (40, 45, and 48°C, applied with a constant water jet) stimulation (spike counts captured during 10 s) of the peripheral receptive field.

Data were captured and analyzed by a Cambridge Electronic Design 1401 interface coupled to a Pentium computer with Spike 2 software (Cambridge, United Kingdom) with poststimulus time histogram and rate functions. After three consecutive stable baseline responses to natural stimuli (values were averaged to give the predrug control values), pharmacologic assessment was carried out (one neuron per animal only).

Drug Administration

Ondansetron (100 μg Zofran; GlaxoSmithKline, Harlow, United Kingdom) was administered *via* topical spinal application to investigate the role of spinal 5HT3 receptors. Pregabalin (10 mg/kg and 30 mg/kg, dissolved in 0.9% saline solution; Pfizer, Inc., Sandwich, United Kingdom) was administered *via* subcutaneous injection for systemic exposure, as used clinically. All drug effects were monitored at 20, 40, and 60 min and are expressed as the mean maximal evoked neuronal response for each dose, unless stated otherwise. Drug effects were not dependent on the control baseline level of activity.

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CRD Model of Visceral Pain

Experiments were conducted on days 7–10 in anesthetized rats. Tracheotomies were performed as previously described.[17] Animals were anesthetized with isoflurane (1.5% v/v) delivered in a gaseous mix of N_2O (66%) and oxygen (33%) before intra-anal insertion (1 cm) of a 7-cm latex balloon tied with silk thread to a cannula perforated throughout a 5-cm tip. An enamel-coated copper electrode was sewn into the right external oblique muscle.

Isoflurane was maintained at 1% v/v during recording. CRD (10–80 mmHg) was produced by inflating the balloon through a pressure amplifier for 30 s with 3 min between each distension and 15 min between each series. Captured signals from muscle activity were amplified, filtered, and displayed on an oscilloscope. Signals were further integrated to produce electromyography values *via* Spike 4 software (Cambridge Electronic Design). Mean electromyographic values evoked during CRD in 30 s (visceromotor responses [VMRs]) were used for further analysis. After pregabalin administration (30 mg/kg), drug effect was analyzed at 20 and 60 min.

Quantitative Polymerase Chain Reaction

We analyzed $\alpha_2\delta$-1, 5HT3A, and μ-opioid receptor (MOR1) messenger ribonucleic acid (mRNA) concentrations in dorsal root ganglia (DRG) for the primary afferents innervating the peripheral receptive field (L4-L5) of WDR neurons. On postoperative days 7–10, DRG from morphine- and saline-treated animals were harvested, pooled as two separate groups, and stored (−80°C). Quantitative polymerase chain reaction (Q-PCR) was performed as described previously.[18] In brief, RNA was extracted from pulverized DRG and isolated using RNeasy columns (QIAGEN, Crawley, United Kingdom) with on-column DNase step. Reverse transcription was carried out on 1-μg RNA using the iScript cDNA Synthesis Kit with random primers (Bio-Rad Laboratories, Inc., Hercules, CA). Q-PCR was performed with an iCycler (Bio-Rad Laboratories, Inc.) using the iQ SYBR Supermix (Bio-Rad Laboratories, Inc.). For each set of primers and for every experiment, a standard curve was generated using a serial dilution of reverse-transcribed RNA from combined samples. The following rat Q-PCR primers were used: GAPDH: 5′-ATGACTCTACCCACGGCAAG-3′ (forward), 5′-CATACTCTGCACCAGCATCTC-3′ (reverse); $\alpha_2\delta$-1: 5′-AGTCTATGTGCCATCAATTAC-3′ (forward), 5′-AGTCATCCTCTTCCATTTCAAC-3′ (reverse); 5HT3A: 5′-AGCCTTGACATCTATAACTTCC-3′ (forward), 5′-TCCGACCTCACTTCTTCTG-3′ (reverse); MOR: 5′-GCCCTCTACTCTATCGTGTGTGTA-3′ (forward), 5′-GTTCCCATCAGGTAGTTGACACTC-3′ (reverse).

Statistics

Analyses were performed using Prism (version 4 for Apple Macintosh OS 10.4; GraphPad Software, San Diego, CA). All data are presented as mean ± SEM unless stated otherwise. Behavioral data were analyzed using the nonparametric Mann–Whitney U test, comparing number of withdrawal responses between saline- and morphine-treated animals. Statistical analysis of electrophysiologic data were as follows: control responses, expressed as number of action potentials, were established for all evoking stimuli before pharmacologic manipulation for each neuron/animal. Differences among naïve, saline, and morphine groups with respect to predrug electrically evoked baseline responses were analyzed using a one-way ANOVA and Bonferroni correction if a significant difference was observed. A two-way ANOVA and Bonferroni correction were used to compare the baseline natural evoked neuronal responses in animal groups. Here, the nature of the stimulus in terms of force or temperature, were factors. A one-way ANOVA and Dunnett *post hoc* multiple comparisons test for significant values were used to evaluate the effect of pregabalin and ondansetron on electrically evoked responses in animal groups. A two-way ANOVA and Bonferroni correction were used to evaluate the effect of pregabalin and ondansetron on evoked responses, again using action potential counts, to natural stimuli in the animal groups. In these latter studies, within each factor, time of drug effect was a level.

Statistical analysis of Q-PCR data were as follows: for each set of primers and for every experiment, a standard curve was generated using a serial dilution of reverse-transcribed RNA from the combined samples. Data were normalized for expression of glyceraldehyde-3-phosphatedehydrogenase mRNA concentration. Data from saline- and morphine-treated animals were then normalized to the mean of the saline group for the corresponding 3 measures (mRNA concentration = 100%) and given as mean ± SEM. Statistical significance of Q-PCR results from both animal groups was determined by unpaired Student *t* tests.

Statistical analysis of VMR data were as follows: electromyography values were integrated with subtracted baselines and postdrug data were normalized for each animal to mean control electromyography values. Normalized electromyographic data were evaluated with the Kolmogorov-Smirnov (Dallal-Wilkinson-Lilliefor corrected *P* value) and D'Agostino and Pearson omnibus normality tests to confirm that data were normally distributed. A two-way ANOVA with repeated measures and Bonferroni correction were used to determine statistical significance throughout the range of CRD pressures between the recording time points for the pregabalin time course in animal groups.

All Data Sets Were Complete With No Lost Data

For all analyses, statistical significance was set at $P < 0.05$ in the text, and $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ in the figures.

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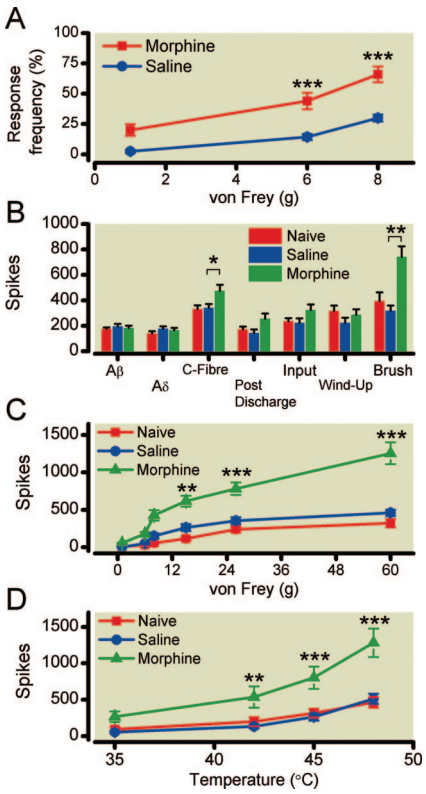

Fig. 1. Mean ± SEM altered behavioral activity and response profile of dorsal horn wide dynamic range spinal neurons after sustained morphine exposure in rats. After 5–8 days of chronic morphine (n = 20) or saline (n = 16) treatment, hind paw withdrawal frequencies to von Frey filaments of increasing force were recorded (*A*). After 7–10 days, evoked neuronal response to electrical (*B*), mechanical (*C*), and thermal (*D*) stimuli were recorded in naive animals (n = 27) as well as those treated with saline (n = 12) and morphine (n = 17). Sustained morphine exposure increased the excitability of spinal neurons to peripherally applied natural stimuli. Based on latency measurements, neuronal responses were subdivided into Aβ-, Aδ-, and C-fibers, or postdischarge. $^{*}P < 0.05$ or $^{**}P < 0.01$ or $^{***}P < 0.001$ *versus* saline group baseline responses.

Results

Behavioral Hypersensitivity and Spinal Neuronal Hyperexcitability Is Evident in Morphine-treated Animals

Animals treated with saline (n = 16) and morphine (n = 20) were tested for signs of behavioral hypersensitivity on days 5–8. Hind paw withdrawal frequencies per animal, per stimulus (1, 6, and 8 g), per day were pooled. A significant increase in hind paw withdrawal frequency was observed in morphine-treated animals (fig. 1A). The median withdrawal frequency significantly increased from 10 to 40 (interquartile range increased from 10 to 40) with 6 g in morphine-treated animals. Application of acetone produced little or no evoked hind paw withdrawal response in either group (data not shown).

Subsequently, naive animals (n = 27) as well as those treated with morphine (n = 17) and saline (n = 12) were used for spinal neuronal electrophysiologic testing. Mean baseline evoked neuronal response to electrical and natural stimulation was not significantly different between control groups ($P > 0.05$). Thus, all results are discussed as a statistical comparison of evoked responses between saline- and morphine-treated animals.

Morphine-treated animals showed a significant increase in C-fiber activity, evoked neuronal response to brush, response to innocuous and noxious mechanical stimuli, and response to noxious temperatures (figs. 1B, C, and D). Modest increases in response to lower mechanical forces and temperatures were observed, but responses to the higher intensities of both modalities were in the order of two- to threefold higher.

Pregabalin Reduces Spinal Neuronal Hyperexcitability in Morphine-treated Animals

Systemic pregabalin (10 mg/kg) was administered and evoked responses were recorded at 20, 40, and 60 min in saline- and morphine-treated animals (both groups, n = 8). After this time, systemic pregabalin (30 mg/kg) was administered and the experimental assay was repeated.

Pregabalin (30 mg/kg) significantly reduced Aβ-, Aδ-, and C-fiber–evoked activity, postdischarge, input, and sensitivities to brush stimulation in morphine- but not saline-treated animals (fig. 2A). Pregabalin (10 mg/kg) significantly reduced evoked neuronal response in morphine- but not saline-treated animals to noxious mechanical and thermal stimuli. Pregabalin (30 mg/kg) produced marginally greater inhibitory effects than the lower dose (figs. 2B, C, D, and E).

Comparing data on mechanical responses (figs. 2B and C), it is noteworthy that pregabalin normalized OIH-enhanced neuronal responses during the whole range. Similar effects of pregabalin were seen for responses to thermal stimuli.

mRNA Concentrations in L4-L5 DRG Are Unchanged in Morphine-treated Animals

We measured and compared the concentration of mRNA for $\alpha_2\delta$-1, 5HT3A, and MOR1 in morphine- and saline-treated animal DRG (L4-L5 pooled, both groups n = 8). No significant changes were observed in mRNA concentrations for any of the proteins analyzed in morphine- *versus* saline-treated animals (fig. 3). Thus pregabalin was effective after OIH, yet its binding site and concentration of MOR1 mRNA were unaltered.

Ondansetron Reduces Spinal Neuronal Hyperexcitability in Morphine-treated Animals

Spinal ondansetron (100 μg) was administered and the evoked response was recorded at 20, 40, and 60 min in morphine-treated animals (n = 8). After this time, systemic pregabalin (10 mg/kg), which previously reduced spinal neuronal hyperexcitability in morphine-treated animals only, was administered and the experimental assay was repeated.

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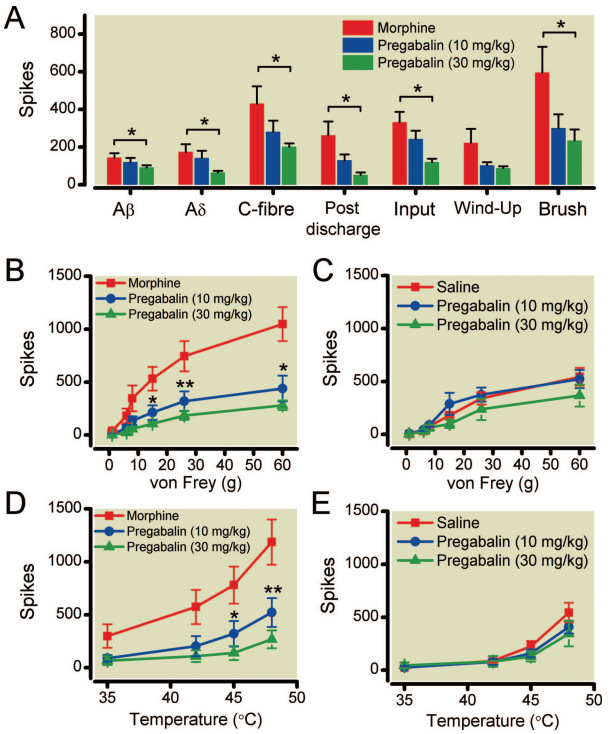

Fig. 2. Mean ± SEM effect of systemic 10 *versus* 30 mg/kg pregabalin on baseline response values of dorsal horn wide dynamic range spinal neurons in saline- and morphine-treated animals (both groups, n = 8). On postoperative days 7–10, the evoked neuronal response to electrical (*A*), mechanical (*B* and *C*), and thermal (*D* and *E*) stimuli were recorded before and after drug treatment in both groups. In animals treated with morphine, but not saline, high-dose (30 mg/kg) pregabalin reduced a subset of electrically evoked responses while low-dose (10 mg/kg) pregabalin reduced the evoked response of spinal neurons to peripherally applied noxious natural stimuli. Based on latency measurements, neuronal responses were subdivided into Aβ-, Aδ-, and C-fibers, or postdischarge. **P* < 0.05 or ***P* < 0.01 *versus* morphine baseline.

Similar to the effects of pregabalin in morphine-treated animals, ondansetron (100 μg) significantly reduced C-fiber–evoked activity, postdischarge, wind up, and sensitivities to brush stimulation (fig. 4A) as well as significantly reducing evoked neuronal response to noxious mechanical and thermal stimuli (figs. 4B and C). However, now, when 10 mg/kg pregabalin was given in the presence of ondansetron, the α-2δ ligand produced no further reduction in evoked response.

Visceral Hyperalgesia Is Evident in Morphine-treated Animals and Pregabalin Reduces Visceral Pain Responses

When compared with saline-treated animals (n = 8), morphine-treated animals (n = 9) developed visceral hyperalgesia (increased sensitivity to noxious visceral stimulation) illustrated by a two-fold increase in the evoked VMR to noxious CRD pressures (fig. 5).

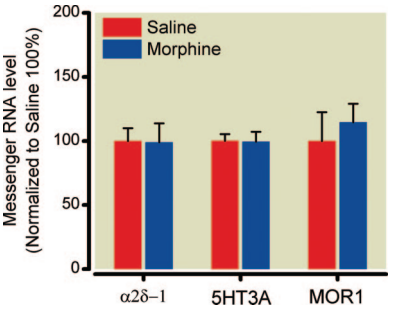

Fig. 3. Mean (percentage of control) ± SEM quantification of $\alpha_2\delta$-1, 5HT3A, and μ-opioid receptor-1 mRNA concentrations in morphine- and saline-treated animal dorsal root ganglia (both groups, n = 8) on postoperative days 7–10 normalized to the mean of the saline group for the corresponding three measures (messenger ribonucleic acid [mRNA] concentration = 100%). There was no significant difference between groups ($P > 0.05$).

Pregabalin (30 mg/kg) administration reduced evoked VMR by noxious CRD pressures in saline- and morphine-treated animals (figs. 6A and B). After 20 min, pregabalin normalized the increased response in morphine-treated ani-

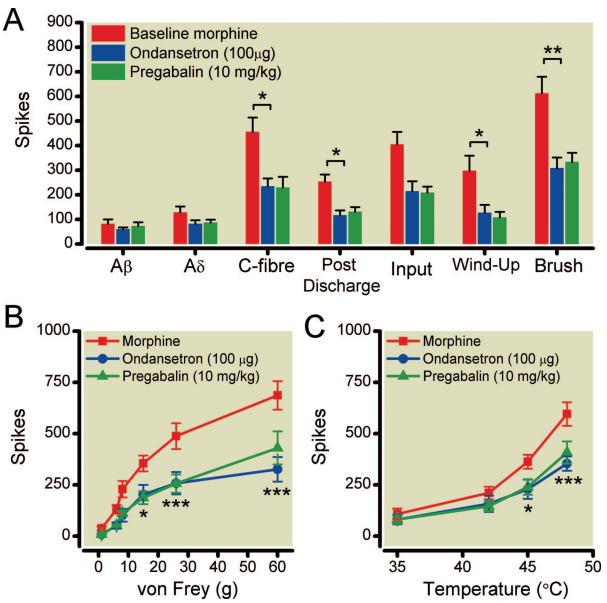

Fig. 4. Mean ± SEM effect of spinal ondansetron (100 μg) and subsequent systemic administration of pregabalin (10 mg/kg) on mean baseline response profiles of dorsal horn wide dynamic range spinal neurons in morphine-treated animals (n = 8). On postoperative days 7–10, evoked neuronal response to electrical (*A*), mechanical (*B*), and thermal (*C*) stimuli were recorded before and after drug treatment. Ondansetron reduced a subset of electrically evoked responses and the evoked response of spinal neurons to peripherally applied noxious natural stimuli. Pregabalin (10 mg/kg) did not further reduce evoked neuronal response to electrical or natural stimuli. Based on latency measurements, neuronal responses were subdivided into Aβ-, Aδ-, and C-fibers, or postdischarge. **P* < 0.05 or ***P* = 0.01 or ****P* < 0.001 *versus* morphine baseline.

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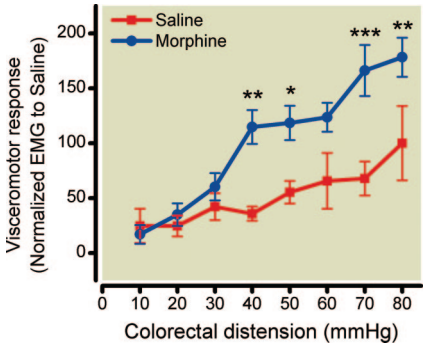

Fig. 5. Mean ± SEM altered visceromotor response (VMR) at noxious colorectal distension (CRD) pressures in morphine-treated animals. On postoperative days 7–10, evoked VMRs to a range of innocuous and noxious CRD pressures were recorded in animals treated with saline (n = 8) *versus* morphine (n = 9). Sustained morphine exposure increased evoked VMR to noxious CRD pressures. $^{*}P < 0.05$ or $^{**}P < 0.01$ or $^{***}P < 0.001$ *versus* saline baseline.

mals to that of the VMR in saline-treated animals. The VMR were not significantly further changed after 60 min (fig. 6C).

There was a wide variation of VMR recorded (figs. 5 and 6), proposed as being due to different batches of animals being used for each set of experiments. All electrophysiologic experimental data for each individual neuron or animal were compared to its own controls, obviating any variation between groups.

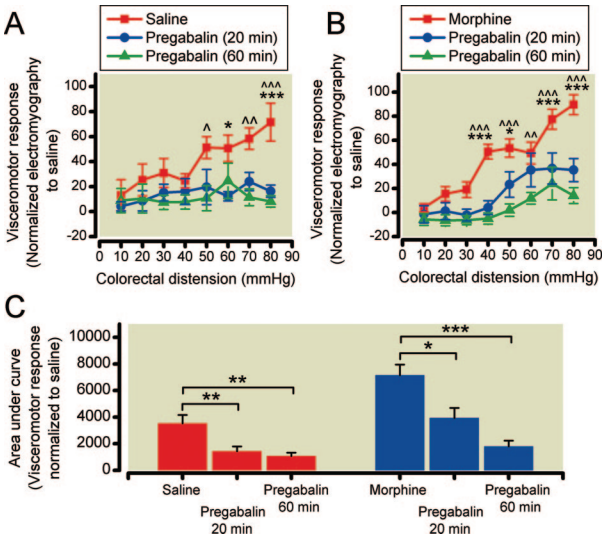

Fig. 6. Mean ± SEM effect of systemic administration of pregabalin (30 mg/kg) on the visceromotor response (VMR) of animals treated with saline (n = 8) and morphine (n = 9). On postoperative days 7–10, evoked response to innocuous and noxious colorectal distension (CRD) pressures were recorded before and after drug treatment. Pregabalin (30 mg/kg) reduced VMR to noxious CRD pressures in animals treated with saline (*A*) and morphine (*B*). After 20 min, pregabalin normalized the increased response in morphine-treated animals to that of VMR in saline-treated animals. VMR was not significantly increased or decreased 60 min after pregabalin treatment (*C*). $^{*}/^{\wedge} P < 0.05$ or $^{**}/^{\wedge\wedge} P < 0.01$ or $^{***}/^{\wedge\wedge\wedge} P < 0.001$, 20-min and 60-min pregabalin treatment, respectively, *versus* baseline.

Discussion

In this study, OIH in rats produced behavioral and spinal neuronal somatic hypersensitivity in the absence of peripheral pathology. We showed that visceral hyperexcitability is also induced and provided evidence that systemic pregabalin normalizes both hypersensitivities. Spinal ondansetron, a 5HT3-receptor antagonist, reduces spinal neuronal hypersensitivity and thereafter attenuates the antinociceptive actions of pregabalin. We propose that, after chronic morphine exposure, a compensatory descending serotonergic pathway from the brainstem increases activity, and that this pathway contributes to a sensitized state in the spinal cord that is permissive for the actions of pregabalin.

Animal models of OIH can be used to model central changes in the presence of normal periphery. This effect can be used to further understand and possibly identify new underlying mechanisms of pain development and maintenance that occur within central pain modulatory circuits after extended opioid treatment. The development and maintenance of OIH is dependent, in part, on neuroadaptive alterations in the pain modulatory circuitry, including increased neurokinin-1 (NK-1) receptor–mediated transmission, spinal dynorphin expression, and hyperactivity of descending facilitatory pathways from higher brain centers.[5–7,19,20]

Morphine exerts its analgesic effects on binding to opioid receptors, and the MOR system plays a pivotal role in OIH development.[21] Peripheral neuropathy alters MOR expression in DRG of neuropathic animals. After peripheral nerve injury, an increase in the proportion of sensory neurons expressing MOR has been observed.[22] Conversely, the number of cells expressing MOR in the DRG of nerve-injured animals was reported drastically reduced.[23] We did not induce peripheral damage in morphine-treated animals, and, despite persistent exposure to morphine, these animals exhibited no change in MOR1 mRNA expression in the DRG. PCR cannot detect nonexpression-related mechanisms of regulation. Decreased modulation of antinociceptive pathways by morphine (as essentially observed in an animal model of OIH) may be the result of progressive desensitization of the MORs after constant ligand exposure.[24] It is worth remembering also that other receptor subtypes are reported to play a crucial role in morphine-induced antinociception.[25] At the level of the spinal cord, opioid receptor agonists produce analgesia by presynaptic attenuation of primary afferent input and postsynaptic inhibition of dorsal horn neurons.[26,27] However, questions relating to where and how opioids initiate plasticity in the CNS that leads to OIH remain unanswered. The role of *N*-methyl-D-aspartic acid receptor–dependent neuronal events at spinal levels after OIH[28] should be further investigated.

Previously, after spinal infusion of opioid agonist and chronic opioid exposure, the characteristics most commonly

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associated with neuropathy including spinal cord hypersensitivity, thermal hyperalgesia, increased spinal dynorphin content, and opioid tolerance were observed in rats in the absence of peripheral pathology.[8,29] Our current study supports and extends these and previous findings that report hypersensitivities to somatic innocuous and noxious thermal and mechanical stimuli after chronic opioid treatment.[20,21,30,31] First, we showed that the altered physiology of deep dorsal horn WDR neurons in morphine animals, whereby increased neuronal activity and behavioral hypersensitivity are both evident, is attenuated by pregabalin.

We then demonstrated that nociceptive sensitization extends to visceral stimuli in morphine animals; specifically, to colonic distension at noxious pressures. We observed sensitized basal VMR to noxious CRD pressures, resulting in dramatically increased magnitude and threshold for visceral pain responses in the CRD model. Pregabalin attenuated graded VMR to CRD in morphine- and saline-treated animals. The analgesic efficacy of pregabalin in naive animals given acute CRD stimuli has been reported previously,[10] an observation that is repeated here with similar nociceptive-specific inhibitory effects on evoked VMR in morphine-treated animals. This finding is reminiscent of the inhibitory action of pregabalin in neuropathy where spinal neuronal and behavioral hypersensitivities are greatly reduced without abolishing the physiologic baseline response.[32,33]

Pregabalin is a starting option in the treatment of many neuropathic pain conditions where the enhanced response of the spinal cord and brain, referred to as central sensitization, develops after sufficient peripheral afferent barrage into the CNS, or by changes in the net balance of descending facilitations and inhibitions from higher centers onto the spinal cord.[34] As we cannot attribute the antinociceptive actions of pregabalin in morphine-treated rats to a state-dependent pathology, we assessed whether up-regulation of the $\alpha_2\delta$-1 accessory subunit of the voltage-gated calcium channel to which pregabalin binds was present in these animals. It is noteworthy that we observed no increase in $\alpha_2\delta$-1 subunit mRNA expression in the DRG-receiving afferents from the peripheral receptive field. Consistent with our findings, previous studies have reported the analgesic efficacy of gabapentinoids in the absence of $\alpha_2\delta$-1 subunit up-regulation[35,36] and in short-term somatic and visceral inflammatory models where $\alpha_2\delta$-1 subunit up-regulation has insufficient time to occur.[37,38] Because the polymodal hypersensitivities of spinal neurons in morphine-treated animals, and the noxious CRD-evoked VMR in saline- and morphine-treated animals, was attenuated by pregabalin in the absence of up-regulation of the $\alpha_2\delta$-1 subunit and peripheral pathology, we conclude that the underlying mechanism determining the analgesic efficacy of pregabalin in morphine-treated animals is not directly comparable to that in chronic pain models. The analgesic actions of pregabalin in our animal model of OIH are most likely related to the modulatory function of, but not to the up-regulation of, voltage-gated calcium channel subunits as observed in neuropathy.

The analgesic efficacy of pregabalin in animal models of chronic pain is experimentally correlated with the injury-dependent interaction of the descending serotonergic system with spinal 5HT3 receptors. Previous studies have shown that, in the absence of peripheral nerve injury, stimulating dorsal horn 5HT3 receptors in normal animals can induce the state-dependent inhibitory actions of the gabapentinoids.[32] We support the premise that enhanced functionality at 5HT3 receptors may be a contributory underlying mechanism in OIH.[19] We showed that the selective 5HT3-receptor antagonist ondansetron attenuated neuronal hyperexcitability in morphine-treated animals, mirroring findings in previous studies where ondansetron inhibited patterns of increased neuronal activity and behavioral patterns of nociception that were, for example, induced by peripheral formalin injection.[39,40] It is noteworthy that, among our morphine-treated animals, when 5HT3 facilitation was blocked by ondansetron, subsequent doses of pregabalin failed to further reduce the spinal neuronal response. This finding lends support to the hypothesis that descending serotonergic circuits also play a key role at spinal levels in regulating the therapeutic actions of pregabalin. We found no increase in 5HT3A mRNA expression in the DRG-receiving afferents from the peripheral receptive field in morphine-treated animals. This finding suggests that spinal neuronal hypersensitivity could be attributed to greater physiologic activity in the descending serotonergic pathways rather than receptor changes. Given the reciprocity between the monoamines 5HT3 and noradrenaline, it is noteworthy that the actions of $\alpha_2\delta$ ligands have also been shown to involve supraspinal and spinal noradrenergic mechanisms.[15]

Thus, there is strong evidence that a spinal-bulbo-spinal loop involving 5HT3 receptor–mediated descending facilitation, which produces central excitability and exists in pain states like neuropathy,[41] also exists in OIH.[19] The hyperexcitability of somatic and visceral responses in morphine-treated animals may reflect the net result of spinal excitability that is enhanced by a positive feedback loop between spinal pronociceptive signaling and alterations in descending modulatory activity, including serotonergic projections from the brainstem.

In summary, we show that pregabalin is active independent of any pathologic state dependency in a visceral and spinal electrophysiology animal model of OIH, where there is no peripheral pathologic trigger. Pregabalin's efficacy may result from proposed central plastic changes in our two experimental models and in the formalin test.[40] This effect may relate to a common factor in visceral hypersensitivity and spinal cord neuronal hyperexcitability, namely normalizing OIH-induced central excitability. Indeed, among humans, using acute capsaicin chemical nociception, Iannetti *et al.*[42] used functional magnetic resonance imaging techniques to show that gabapentinoids have a state-dependent action for

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modulation of brainstem activation only in the presence of central sensitization. It is therefore possible that a threshold level of central excitability is a prerequisite and is sufficient for pregabalin to exert analgesic actions when the periphery is undamaged.

Common mechanisms can therefore contribute to a variety of experimental pain responses and also to their therapy. Spinal NK-1R–expressing neurons are at the origin of spinal-bulbo-spinal loops that engage the brainstem and 5HT3 receptors. Thus, ablation of these neurons and blocking this serotonergic drive reduces responses to formalin, neuropathy, and the manifestation of OIH—as well as the antihyperalgesic actions of pregabalin.[14,19,32,41]

OIH deserves attention because morphine remains the "gold standard" analgesic. Its long-term use in the clinic can be limited, however, by any ensuing paradoxical heightened pain sensations.[43–45] In the presence of normal periphery, understanding the central changes that must occur for hyperalgesia to manifest after chronic opioid treatment is paramount to optimizing the use of morphine. This knowledge would allow combination therapies to be offered to patients receiving long-term morphine treatment. We provide evidence that pregabalin reduces neuronal hyperexcitability and visceral hypersensitivity after chronic morphine exposure in rats. Theoretically, pregabalin could be seen as a viable combination therapy option in the treatment of patients with OIH because it reduced morphine-induced hyperexcitability of somatic and visceral responses. It is noteworthy that gabapentin and morphine display supra-additive neuronal inhibitory effects and analgesia after acute administration in animals and more chronically in human studies of neuropathy.[46,47] Thus, in OIH, the common direction of effect of these drugs diverges so that the $\alpha_2\delta$-1 receptor ligand can still reduce and normalize enhanced responses produced by the opioid.

Finally, the mechanisms that underlie OIH—namely a plethora of linked central changes—may shed light on events that could relate to idiopathic diffuse pain states that include somatic and visceral hypersensitivity. Responses to both modalities became hypersensitized in morphine-treated animals. We find it an encouragement to future research efforts that $\alpha_2\delta$-1 receptor ligands are offered as an emerging therapy in the treatment of diffuse pain states, such as irritable bowel syndrome and fibromyalgia, which possibly represent additional cases of central sensitization.[48,49]

The authors thank Wahida Rahman, Ph.D. (Postdoctoral Researcher, Department of Neuroscience, Pharmacology and Physiology, University College London, London, United Kingdom), for extraction of rat dorsal root ganglia.

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