Research Paper

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Diffuse noxious inhibitory controls and nerve injury: restoring an imbalance between descending monoamine inhibitions and facilitations

Kirsty Bannister*, Ryan Patel, Leonor Goncalves, Louisa Townson, Anthony H. Dickenson

Abstract

Diffuse noxious inhibitory controls (DNICs) utilize descending inhibitory controls through poorly understood brain stem pathways. The human counterpart, conditioned pain modulation, is reduced in patients with neuropathy aligned with animal data showing a loss of descending inhibitory noradrenaline controls together with a gain of 5-HT_3 receptor-mediated facilitations after neuropathy. We investigated the pharmacological basis of DNIC and whether it can be restored after neuropathy. Deep dorsal horn neurons were activated by von Frey filaments applied to the hind paw, and DNIC was induced by a pinch applied to the ear in isoflurane-anaesthetized animals. Spinal nerve ligation was the model of neuropathy. Diffuse noxious inhibitory control was present in control rats but abolished after neuropathy. α_2 adrenoceptor mechanisms underlie DNIC because the antagonists, yohimbine and atipamezole, markedly attenuated this descending inhibition. We restored DNIC in spinal nerve ligated animals by blocking 5-HT_3 descending facilitations with the antagonist ondansetron or by enhancing norepinephrine modulation through the use of reboxetine (a norepinephrine reuptake inhibitor, NRI) or tapentadol (μ-opioid receptor agonist and NRI). Additionally, ondansetron enhanced DNIC in normal animals. Diffuse noxious inhibitory controls are reduced after peripheral nerve injury illustrating the central impact of neuropathy, leading to an imbalance in descending excitations and inhibitions. Underlying noradrenergic mechanisms explain the relationship between conditioned pain modulation and the use of tapentadol and duloxetine (a serotonin, NRI) in patients. We suggest that pharmacological strategies through manipulation of the monoamine system could be used to enhance DNIC in patients by blocking descending facilitations with ondansetron or enhancing norepinephrine inhibitions, so possibly reducing chronic pain.

Keywords: Diffuse noxious inhibitory controls, Adrenoceptors, Monoamines, Descending facilitations, Conditioned pain modulation, Descending inhibitions, Dorsal horn, In vivo electrophysiology, Neuropathic pain

1. Introduction

Le Bars et al.[20] introduced the scientific community to diffuse noxious inhibitory controls (DNICs) whereby application of strong pain to one part of the body inhibits pain in multiple remote body regions. Diffuse noxious inhibitory controls are a unique form of endogenous analgesia, partly opioid,[18] in which the activity of trigeminal and spinal convergent wide-dynamic-range (WDR) neurons is inhibited through descending pathways.[21] In terms of descending modulation of the spinal cord, important pathways involve the dorsolateral funiculus comprising the periaqueductal grey, locus coeruleus (LC), and rostral ventromedial medulla (RVM) projections to the spinal cord.[32] Both serotonergic and noradrenergic projection neurons from these regions can lead to inhibitory and/or excitatory effects on pain modulation.[2] An aim of this study was to define the spinal pharmacology of pathways that subserve DNIC with an emphasis on these monoamine systems.

A separation between the DNIC system and the traditionally viewed endogenous descending inhibitory control system through the dorsolateral funiculus (as outlined above) was suggested following a series of lesioning experiments that showed that supraspinal structures including the periaqueductal grey, RVM, and LC were not involved directly in DNIC.[6–8] Rather, the pain modulatory action observed involves, to some extent, activation of descending inhibitory pathways from the subnucleus reticularis dorsalis (SRD) to the dorsal horn of the spinal cord.[9] However, inactivating the RVM was shown to reinstate DNIC in chronic morphine-treated animals[25] suggestive of complex brain stem interconnections ultimately impacting on descending monoamine systems. Physiopathological events could alter the opposing monoamine controls mediated by noradrenergic and serotonergic systems[2] that now may compromise inhibition and favour facilitation, so perturbing DNIC.

Conditioned pain modulation (CPM) is the term used to describe psychophysical paradigms in which a distant painful conditioning stimulus is used to affect a test stimulus and so is the human counterpart of DNIC.[34] Conditioned pain modulation has been related to persistent postsurgical pain. In neuropathic pain and other pain patients, CPM is reduced[34] indicative of altered descending modulation in these patients. Importantly, reduced CPM was predictive of the actions of duloxetine, a serotonin–norepinephrine reuptake inhibitor (SNRI), in patients with painful

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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diabetic neuropathy[36] and restored by tapentadol, a centrally acting analgesic that is both a μ-opioid receptor agonist and a norepinephrine reuptake inhibitor (MOR–NRI).[24] Diffuse noxious inhibitory controls and CPM offer an opportunity to link preclinical studies with patients. To back-translate from these clinical observations, we investigate the pharmacology of DNIC. The supraspinal circuitry for DNIC has not been convincingly defined. It is hypothesized that noradrenergic and serotonergic inhibitory and facilitatory controls acting on spinal cord WDR neurons subserve and/or influence DNIC in the normal situation. Given that in spinal nerve ligated (SNL) animals, it is known that there is a downregulation of α_2 adrenoceptor (AR)-mediated inhibitions and an upregulation of 5-HT_3 receptor-mediated facilitations,[26,30] it might be expected that these changes lead to a disruption of DNIC after neuropathy; we investigate whether restoration of the normal balance in descending controls could restore DNIC.

2. Methods

2.1. Animals

Male Sprague-Dawley rats (250-300 g; UCL Biological Services, London, United Kingdom) were used for electrophysiological experiments. Animals were group housed on a 12:12-hour light–dark cycle. Food and water were available ad libitum. All procedures described were approved by the Home Office and adhered to the Animals (Scientific Procedures) Act 1986. Every effort was made to reduce animal suffering and the number of animals used in accordance with the IASP ethical guidelines.[37]

2.2. Spinal nerve ligation surgery

Spinal nerve ligation surgery was performed as described previously.[17] Rats (120-140 g) were maintained under 2% vol/vol isoflurane anaesthesia delivered in a 3:2 ratio of nitrous oxide and oxygen. Under aseptic conditions, a paraspinal incision was made and the left tail muscle excised. Part of the L5 transverse process was removed to expose the L5 and L6 spinal nerves, which were then isolated with a glass nerve hook (Ski-Ry Ltd, London, United Kingdom) and ligated with a nonabsorbable 6-0 braided silk thread proximal to the formation of the sciatic nerve. The surrounding skin and muscle were closed with absorbable 3-0 sutures. Sham surgery was performed in an identical manner omitting the ligation step. All rats were monitored for normal behaviours (grooming and mobility) and weight gain after surgery.

2.3. Electrophysiology

In vivo electrophysiology experiments were conducted on postoperative days 14 to 18 (sham and SNL-operated animals) on weight-/age-matched naive rats as previously described.[31] Briefly, animals were anesthetised and maintained for the duration of the experiment with isofluorane (1.5%) delivered in a gaseous mix of N_2O (66%) and O_2 (33%). A laminectomy was performed to expose the L4 and L5 segments of the spinal cord. Extracellular recordings were made from deep dorsal horn neurons (laminae V–VI) using parylene-coated tungsten electrodes (A-M Systems, Sequim, WA). All the neurons recorded were WDR and responded to natural stimuli including brush and low and high intensity mechanical and thermal stimuli in a graded manner with coding of increasing intensity.

The peripheral receptive field was stimulated using punctate mechanical stimuli (von Frey [vF] filaments: 8, 26, and 60*g*), and the number of action potentials fired in 5 seconds was recorded. Data were captured and analysed by a CED 1401 interface coupled to a Pentium computer with Spike2 software (Cambridge Electronic Design; rate functions).

Three baseline responses to mechanical stimuli as detailed above were characterised for each neuron before DNIC and subsequent pharmacological assessment (a drug study was conducted on 1 neuron per animal only).

2.4. Diffuse noxious inhibitory control study design

Extracellular recordings were made from 1 WDR neuron per animal by stimulating the hind paw peripheral receptive field and then repeating in the presence of ear pinch. The number of action potentials fired in 5 seconds was recorded for each test. Baseline responses were calculated from the mean of 2 trials. Each trial consisted of 3 consecutive stable responses to 8, 26, and 60*g* von Frey filaments applied to the hind paw (where all neurons met the inclusion criteria of <10% variation in action potential firing for all mechanically evoked neuronal responses). This was then followed by consecutive responses to the same mechanical stimuli (8, 26, and 60*g* von Frey filaments) in the presence of DNIC. Precisely, DNIC was induced using a noxious ear pinch (15.75 × 2.3 mm Bulldog Serrefine; InterFocus, Linton, United Kingdom) on the ear ipsilateral to the neuronal recording, whilst concurrent to this, the peripheral receptive field was stimulated using the von Frey filaments listed. Diffuse noxious inhibitory control was quantified as an inhibitory effect on neuronal firing during ear pinch. A 1-minute nonstimulation recovery period was allowed between each test in the trial. After this, for predrug neuronal recordings, a 10-minute nonstimulation recovery period was allowed before the entire process was repeated and data for control trial number 2 were collected.

2.5. Drug administration

After collection of predrug baseline control data as outlined in the Diffuse noxious inhibitory control study design section, the drugs listed below were administered (1 drug per neuron). Each individual drug dose effect was followed for up to 60 minutes with tests performed at 2 time points (10 and 40 minutes). For each time point, a trial consisted of (1) 3 consecutive stable responses to 8, 26, and 60*g* von Frey filaments (where all neurons met the inclusion criteria of <10% variation in action potential firing for all mechanically evoked neuronal responses) followed by (2) consecutive responses to 8, 26, and 60*g* von Frey filaments with concurrent ear pinch. For postdrug DNIC effects, maximal changes from predrug DNIC responses are presented in the graphs for **Figures 1–4**.

The following drugs were used: Atipamezole is an α_2-AR antagonist[26] (100 μg; Sigma-Aldrich, Gillingham, United Kingdom, dissolved in 97% normal saline, 2% Cremophor [Sigma], 1% dimethyl sulfoxide [DMSO; Sigma] vehicle); atipamezole was administered topically to the spinal cord in 50 μL volumes. Yohimbine is an α_2-AR antagonist[11] (5 mg/kg; Sigma, dissolved in a vehicle of 85% normal saline, 10% Cremaphor, 5% DMSO); yohimbine was administered subcutaneously. Ondansetron is a 5-HT_3 receptor antagonist[30] (100 μg; Claris Lifesciences, Cheshire, United Kingdom, dissolved in a vehicle of normal saline); ondansetron was administered topically to the spinal cord in 50 μL volumes. Reboxetine mesylate is an NRI[16] (100 μg; Tocris, Abingdon, United Kingdom, dissolved in 97% normal saline, 2% Cremaphor, 1% DMSO vehicle); ondansetron was administered topically to the spinal cord in 50 μL volumes. Tapentadol is an NRI and MOR agonist[5] (1 mg/kg, a gift from

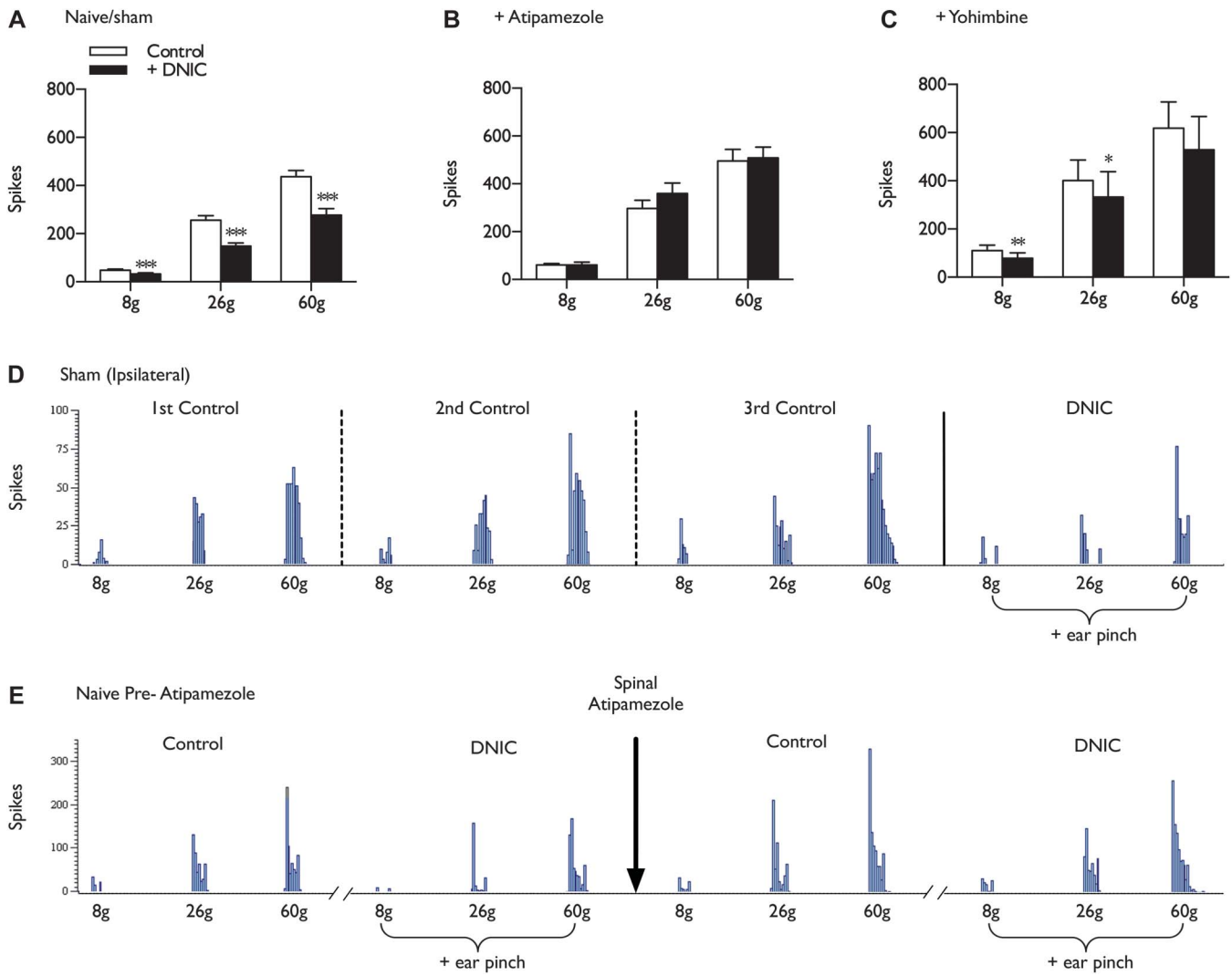

Figure 1. The effect of diffuse noxious inhibitory control (DNIC) activation on response profiles of deep dorsal horn wide-dynamic-range neurons in naive and sham-operated rats before (n = 18, data pooled) and after either intrathecal application of atipamezole (100 μg, n = 6) or subcutaneous yohimbine (5 mg/kg, n = 6) is shown. All data are presented as mean ± SEM.[14] Here, the DNIC effect is expressed as the maximal evoked change in neuronal response. In all experimental groups, the evoked responses to mechanical stimuli were recorded before and after activation of DNIC. A noxious ear pinch ipsilateral to the neuronal receptive field significantly reduced the excitability of spinal neurons to simultaneous peripherally applied non-noxious and noxious stimuli in naive and sham-operated animals (A). In the presence of spinal atipamezole, a noxious ear pinch now had no statistically significant effect on the excitability of spinal neurons to simultaneous peripherally applied non-noxious and noxious stimuli (B). In contrast, in the presence of systemic yohimbine, a noxious ear pinch partially inhibited neuronal responses to noxious mechanical stimulation compared with baseline (C). The first representative trace shows 3 control responses to von Frey filaments and a response profile after the simultaneous application of a noxious ear pinch. There is a statistically significant comparative reduction in neuronal action potential firing after activation of DNIC in naive and sham-operated rats (D). This effect is reversed after treatment with atipamezole. The second representative trace shows 1 example of a predrug control and then the response profile after the simultaneous application of a noxious ear pinch, and then 1 example of a postdrug control and the response profile after the simultaneous application of a noxious ear pinch (E). Traces represent single-unit recordings. Columns represent number of spikes per second. Note that for the raw data in all figures, the range of the vertical scale varies amongst the experimental groups for clarity of illustration. Significant differences from baseline response: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Grunenthal); tapentadol was dissolved in normal saline and administered subcutaneously.

2.6. Statistical analyses

Statistical analyses were performed using SPSS v22 (IBM, Armonk, NY). All data plotted in **Figures 1–4** represent mean ± SEM. Statistical differences in the neuronal responses observed after ear pinch and/or after drug administration were determined using a 2-way repeated-measures analysis of variance (RM-ANOVA) with Bonferroni post hoc test. The Mann–Whitney *U* test followed by Bonferroni correction was used for analysis of percentage changes (as depicted in **Fig. 4**). Asterisks denote statistically significant differences ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

3. Results

3.1. Diffuse noxious inhibitory controls are present in naive and sham-operated rats and this inhibitory effect is reversed by atipamezole and yohimbine

Throughout this study, DNICs were induced by a noxious ear pinch applied to the ear ipsilateral to the neuron being recorded. The presence of DNIC was confirmed in all neurons by a concurrent reduction in deep dorsal horn WDR neuronal firing to stimulation of the hind paw peripheral receptive field. The

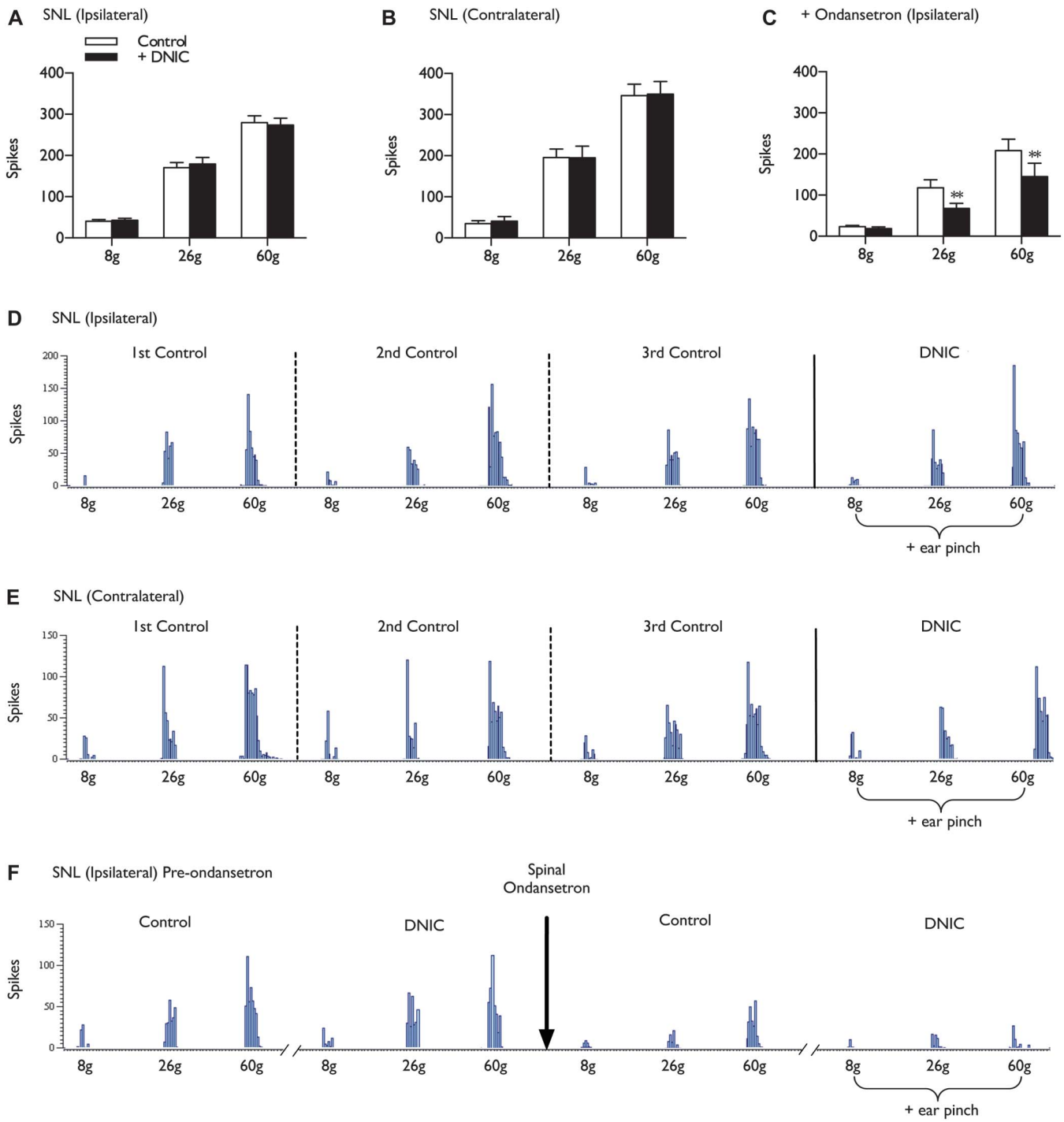

Figure 2. The effect of diffuse noxious inhibitory control (DNIC) activation on response profiles of deep dorsal horn wide-dynamic-range neurons in spinal nerve ligated (SNL) animals (n = 18) before and after treatment with intrathecal ondansetron (100 μg) is shown. All data are presented as mean ± SEM.[14] Here, the DNIC effect is expressed as the maximal evoked change in neuronal response. In all experimental groups, the evoked responses to mechanical stimuli were recorded before and after activation of DNIC. A noxious ear pinch ipsilateral to the neuronal receptive field had no statistically significant effect on the excitability of spinal neurons to peripherally applied stimuli in SNL rats, neither on the ipsilateral nor on the contralateral side (A and B, respectively). However, in the presence of ondansetron after a noxious ear pinch, the excitability of spinal neurons to simultaneous peripherally applied mechanical stimuli was significantly reduced (C). The first 2 representative traces show 3 control responses to von Frey filaments and a response profile after the simultaneous application of a noxious ear pinch. There is no statistically significant reduction in neuronal action potential firing after activation of DNIC in SNL rats on either the ipsilateral or the contralateral side (D and E, respectively). This effect is reversed after treatment with ondansetron. The third representative trace shows 1 example of a predrug control and then the response profile after the simultaneous application of a noxious ear pinch, and then 1 example of a postdrug control and the response profile after the simultaneous application of a noxious ear pinch (F). Traces represent single-unit recordings. Columns represent number of spikes per second. Significant differences from baseline response: $^{**}P < 0.01$.

magnitude of DNIC was examined in naive (n = 12) and sham-operated (examined 14 days after sham SNL surgery, n = 6) animals. Because no difference in the level of neuronal inhibition upon activation of DNIC was observed between naive and sham-operated animals, data from these groups were pooled throughout. Activation of DNIC by heterotopic application of an

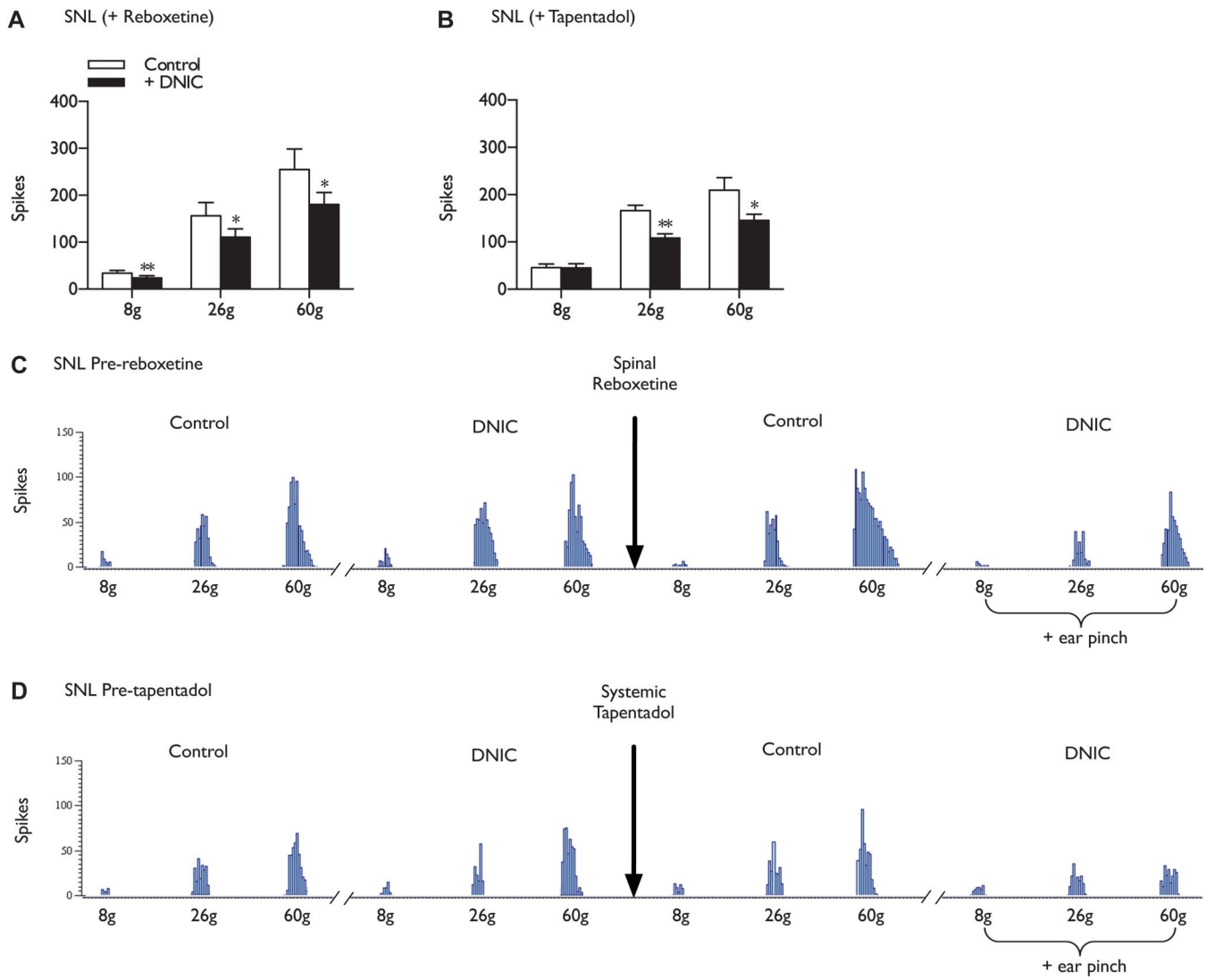

Figure 3. The effect of intrathecal reboxetine (50 μg) or systemic tapentadol (1 mg/kg) on the response profiles of wide-dynamic-range spinal neurons in spinal nerve ligated rats (n = 6 for both groups) is shown. Here, drug effect is expressed as the mean maximal evoked change in neuronal response. In both animal groups, the evoked responses to mechanical stimuli were recorded before and after activation of diffuse noxious inhibitory controls. In the presence of reboxetine after a noxious ear pinch ipsilateral to the neuronal receptive field, the excitability of spinal neurons to simultaneous peripherally applied non-noxious and noxious stimuli was significantly reduced (A). In the presence of tapentadol, after a noxious ear pinch ipsilateral to the neuronal receptive field, the excitability of spinal neurons was significantly reduced to simultaneous peripherally applied noxious stimuli only (B). The representative traces show 1 example of a predrug control and then the response profile after the simultaneous application of a noxious ear pinch, and then 1 example of a postdrug control and the response profile after the simultaneous application of a noxious ear pinch. There is a statistically significant reduction in firing after activation of diffuse noxious inhibitory controls in spinal nerve ligated rats treated with reboxetine (C) and tapentadol (D). Traces represent single-unit recordings. Columns represent number of spikes per second. Significant differences from baseline response: $^{*}P < 0.05$, $^{**}P < 0.01$.

ear pinch (conditioning stimulus) significantly and dramatically reduced the WDR neuronal response to non-noxious and noxious mechanical stimuli in both animal groups (34, 43, and 37% inhibition to 8, 26, and 60*g* vF, respectively; $P < 0.001$ for all forces; 2-way RM-ANOVA; $P < 0.001$, $F_{(1,17)} = 316.03$) (**Fig. 1A**, n = 18). The magnitude of DNIC was then examined in naive rats after spinal application of atipamezole (100 μg, n = 6) or subcutaneous injection of yohimbine (5 mg/kg, n = 6). Remarkably now, in the presence of the selective α_2-AR antagonist atipamezole, no reduction in WDR neuronal response to mechanical stimuli was observed upon application of the conditioning stimulus (0, +20, and +12% inhibition to 8, 26, and 60*g* vF, respectively; $P > 0.05$ for all forces; 2-way RM-ANOVA; $P > 0.05$, $F_{(1,5)} = 5.83$) (**Fig. 1B**), thereby demonstrating a complete blockade of DNIC. Meanwhile, systemic yohimbine, another α_2-AR antagonist, clearly but partially prevented this inhibitory effect (29, 17, and 15% inhibition to 8, 26, and 60*g* vF, respectively; this preventative effect was significant for 8*g* and 26*g* von Frey stimulation only; $P < 0.001$ and $P < 0.05$, respectively; 2-way RM-ANOVA; $P < 0.05$, $F_{(1,5)} = 135.56$) (**Fig. 1C**).

3.2. Diffuse noxious inhibitory controls are not present in spinal nerve ligated rats but are revealed after spinal application of ondansetron

The magnitude of DNIC was examined in unilaterally nerve ligated rats 14 days after SNL surgery on responses evoked from the side of the injury (the ipsilateral side). In sharp contrast to naive and sham-operated animals, there was no reduction in WDR neuronal response to mechanical stimulation during ear pinch in SNL

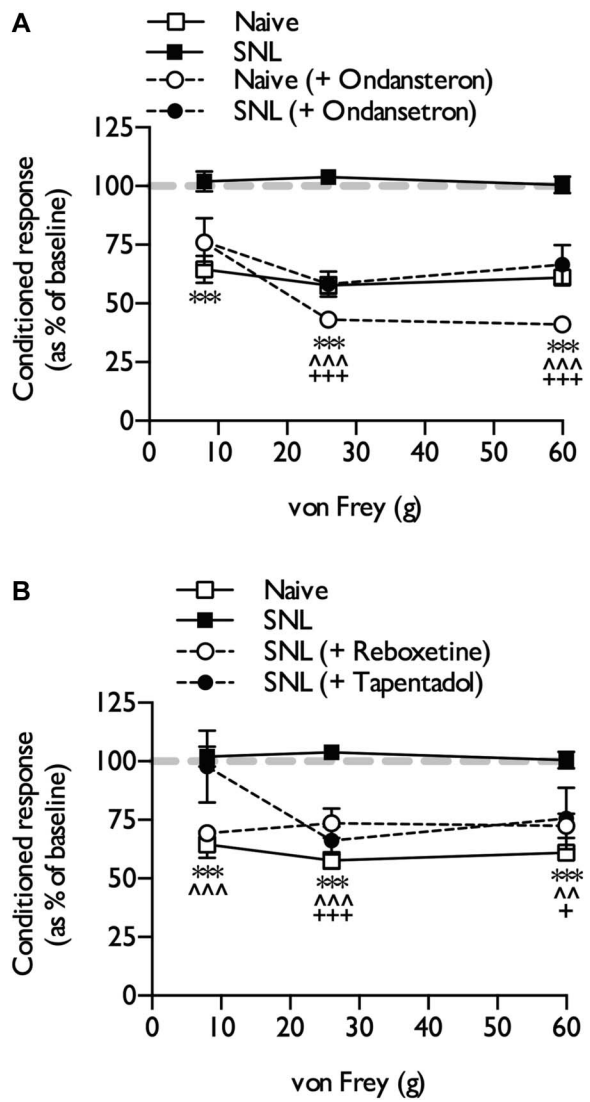

Figure 4. Conditioned response (as a percentage of the baseline) comparisons of the evoked neuronal response to non-noxious and noxious mechanical stimulation in spinal nerve ligated (SNL) and naive animal groups are shown. After treatment with ondansetron, there is an inhibitory effect upon activation of diffuse noxious inhibitory controls (DNIC) in SNL animals that produces an inhibition comparable with that observed in naive animals. Furthermore, the magnitude of DNIC is significantly enhanced in naive animals after treatment with ondansetron. * represents difference between SNL and naive; ^ represents difference between SNL and SNL (ondansetron); + represents difference between naive and naive (ondansetron) (A). Mann-Whitney *U* test followed by Bonferroni correction, ***$P < 0.001$. Treatment with reboxetine and tapentadol in SNL animals also results in recovery of the inhibitory effect upon activation of DNIC to either all mechanical stimuli (reboxetine) or noxious mechanical stimuli only (tapentadol). * represents difference between SNL and naive; ^ represents difference between SNL and SNL (reboxetine); + represents difference between SNL and SNL (tapentadol) (B). Mann-Whitney *U* test followed by Bonferroni correction, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

animals (+6, +3, and 3% inhibition to 8, 26, and 60*g* vF, respectively; $P > 0.05$ for all forces; 2-way RM-ANOVA; $P > 0.05$, $F_{(1,16)} = 1.82$) (**Fig. 2A**, n = 18). In addition, DNIC was absent on the uninjured contralateral side (+14, 3, and 0% inhibition to 8, 26, and 60*g* vF, respectively; $P > 0.05$ for all stimuli; 2-way RM-ANOVA; > 0.05, $F_{(1,4)} = 0.106$) revealing a bilateral loss of this inhibitory control in neuropathic rats (**Fig. 2B**, n = 6). For the remainder of the article, all SNL results discussed relate to neuronal recordings on the ipsilateral side of the ligation.

The degree of inhibition produced by DNIC was next examined in SNL rats after spinal application of the 5-HT_3 receptor antagonist ondansetron (100 μg, n = 6). As described above, DNIC was abolished by the neuropathy but interestingly now, in the presence of ondansetron, which would block descending facilitation, there was a significant and dramatic reduction of WDR neuronal responses to noxious mechanical stimuli upon simultaneous ear pinch, which achieved levels of inhibition comparable with that observed in naive and sham-operated animals (43% and 30% inhibition to 26*g* and 60*g* vF, respectively, in SNL animals; $P < 0.01$ for all forces; 2-way RM-ANOVA; $P < 0.01$, $F_{(1,5)} = 37.64$, almost identical to the 43% and 37% inhibition to 26*g* and 60*g* vF in the naive control data group) (**Figs. 2C and 4**).

3.3. Diffuse noxious inhibitory controls are revealed in spinal nerve ligated rats after spinal application of reboxetine or after systemic administration of tapentadol

Having shown that blocking 5-HT_3 receptor-mediated facilitations could restore DNIC in SNL animals, we now attempted to enhance inhibitory controls. The degree of inhibition produced by DNIC was examined in SNL rats after spinal application of reboxetine, an NRI (50 μg, n = 6), or after systemic administration of tapentadol (1 mg/kg, n = 6), which has a dual mode of action as an agonist of MOR and NRI. Remarkably, in the presence of reboxetine, the conditioning stimulus now induced a significant reduction in WDR neuronal responses to innocuous and noxious mechanical stimuli in SNL animals (29, 29, and 29% inhibition to 8, 26, and 60*g* vF, respectively; $P < 0.001$, $P < 0.01$, and $P < 0.01$, respectively; 2-way RM-ANOVA; $P < 0.05$, $F_{(1,5)} = 12.77$) (**Fig. 3A**). Tapentadol also exerted similar effects in reinstating conditioned inhibition of WDR neuronal responses in SNL animals, but to the noxious mechanical stimuli only (35% and 24% inhibition to 26*g* and 60*g* vF, respectively; $P < 0.01$ and $P < 0.05$, respectively; 2-way RM-ANOVA; $P < 0.05$, $F_{(1,5)} = 14.32$). Responses to 8*g* vF were only reduced by 5% (**Fig. 3B**). The recovery of DNIC by these pharmacological manipulations in both cases was only slightly less than that seen in the control groups (**Fig. 4**).

3.4. Conditioned responses as a percentage of control values affirm that the pharmacological restoration of diffuse noxious inhibitory controls in spinal nerve ligated animals produces comparable levels of neuronal inhibition to those observed in naive animals

Conditioned (DNIC) neuronal responses were plotted as a percentage of preconditioned control responses. Here, we depict in **Figure 4** the significant changes presented in **Figures 1–3**, now expressed as percentage inhibitions to allow direct and simple comparisons of the effect of the drugs on DNIC. In naive and sham-operated rats, activation of DNIC significantly reduced WDR neuronal responses to 8, 26, and 60*g* vF to 66, 57, and 63% of control responses, respectively ($P < 0.001$ for all forces). In marked contrast in SNL rats, no significant effect of DNIC was seen and conditioned evoked neuronal responses were nearly identical to preconditioned control values (100, 105, and 99% of control responses to 8, 26, and 60*g* vF, respectively). However, after spinal application of ondansetron in SNL rats, the degree of inhibition produced by activation of DNIC was comparable with that observed in naive and sham-operated animals (80, 57, and 70% of control responses to 8, 26, and 60*g* vF, respectively). Furthermore, now there was a significant decrease in response to

26*g* and 60*g* vF ($P < 0.001$ for both forces). Not only did ondansetron restore DNIC after neuropathy but also in naive animals, the 5-HT_3 receptor antagonist augmented DNIC-mediated inhibitions of evoked neuronal responses. This enhancement was greater for noxious mechanical stimuli (43% and 41% of control responses to 26*g* and 60*g* vF, respectively; $P < 0.001$ for both forces) (**Fig. 4A**). The NRI reboxetine also dramatically enhanced DNIC-evoked inhibition of neuronal activity in SNL rats (74, 73, and 74% of control responses to 8, 26, and 60*g* vF, respectively; $P < 0.001$, $P < 0.001$, and $P < 0.01$, respectively), as did tapentadol, although this effect was limited to noxious mechanical stimuli only (70% and 75% of control responses to 26*g* and 60*g* vF, respectively; $P < 0.001$ and $P < 0.05$, respectively) (**Fig. 4B**).

3.5. Baseline control values are altered according to the pharmacology in question

The baseline neuronal responses were modulated by some of the drugs used, in keeping with the findings from our previous studies.[26,30] Generally, blocking the inhibitory α_2 AR lead to an increase in neuronal responses in control animals, whereas ondansetron, by attenuating 5-HT_3 receptor-mediated facilitations through its action as a 5-HT_3 receptor antagonist, reduced activity. These data are not shown but are illustrated in the examples of spike traces before and after drug treatment in **Figures 1E and 2F**. In the case of tapentadol, we tested its ability to restore DNIC using a low dose that only has minimal effects on control-evoked responses.[5] Across the various pharmacological and electrophysiological conditions, the level of baseline activity was unrelated to the magnitude of DNIC. For example, as previously described,[5] there were reduced control responses in the SNL animals compared with the control animals, explicable in terms of a reduced input onto these neurons after peripheral neuropathy.[5] Even so, despite a reduced baseline response in SNL animals, DNIC was completely ineffective here.

4. Discussion

We provide a pharmacological basis for DNIC at the spinal level and its alteration after neuropathy. We show unequivocally that DNICs are active in naive and sham-operated animals evidenced by a significant decrease in deep dorsal horn neuronal firing to mechanical stimulation of the hind paw during concurrent noxious ear pinch. Diffuse noxious inhibitory controls were totally absent in SNL animals. Furthermore, DNICs were abolished in control animals upon administration of α_2-AR antagonists atipamezole or yohimbine and revealed in SNL animals upon administration of 5-HT_3 receptor antagonist ondansetron, or by increasing spinal noradrenergic content using reboxetine (NRI) or tapentadol (MOR–NRI). Our data support preexisting evidence of a disruption in the balance between inhibitory (α_2 AR-mediated) and facilitatory (serotonin receptor–mediated) descending monoamine transmission to the dorsal horn of the spinal cord after neuropathy.[26,30] We extend these findings by suggesting that this imbalance influences the expression of DNIC. Thus, after neuropathy, the α_2 AR and 5-HT_3 receptor changes impact on baseline responses to applied stimuli and also compromise the ability to induce DNIC. Normally, these systems exert bidirectional controls on evoked responses, but the application of a second stimulus is able to activate a noradrenergic control that overrides the excitatory events, both local and descending, to trigger DNIC. This is lost in the SNL animals but can be restored by attenuating the now dominant 5-HT_3 receptor-mediated facilitation. In normal animals, reducing facilitations with ondansetron further enhances DNIC.

The final spinal action of DNIC inhibits convergent neurons of the dorsal horn through a descending noradrenergic pathway. Unlike atipamezole, yohimbine partially reversed the inhibitory actions of DNIC in control animals. This difference could arise from higher local spinal concentrations of atipamezole. While reboxetine reinstated DNIC in SNL animals to a comparable level to control animals, tapentadol restored DNIC on noxious peripheral stimulation but not to the lowest force of 8*g* (**Fig. 4B**). Tapentadol is a dual-acting analgesic through MOR–NRI mechanisms. The difference observed may be due to the opioid mechanisms of tapentadol preferentially acting on the higher forces alongside the noradrenergic actions. Ondansetron enhanced the inhibitory effect of DNIC in control animals and reinstated DNIC in SNL animals. Blocking excitations or enhancing inhibitions in neuropathy were equally effective and restored DNIC to levels seen in control animals.

Under the conditions of our study, the inhibitory effects of DNIC were about 35%, somewhat similar to that seen in humans with CPM.[36] The translation of DNIC acting on deep dorsal horn neurons to humans is supported by the fact that these spinal neurons code the intensity and spatial features of stimuli under the same anaesthetic conditions, in a manner remarkably parallel to human psychophysics.[28] Indeed, a recent study in patients with diabetic neuropathy showed that tapentadol restores CPM, exactly as we found here.[24]

Descending controls appear to lack strict somatotopy. Diffuse noxious inhibitory controls were absent ipsilaterally and contralaterally in neuropathic rats, revealing a bilateral loss of this inhibitory control. After nerve injury, noradrenergic tone may control nociception and oppose the spread of sensitization at the spinal level to the other side.[16] This suggests that descending controls are changed on a global level, and this is in accord with the whole-body receptive fields of neurons in SRD, likely to be an important brain stem relay in DNIC.[7,9,33] The pharmacology of this nucleus remains undefined, but our data suggest links to norepinephrine brain stem zones. Recent imaging studies support the idea that very similar brain stem structures are activated during pain and hyperalgesia in human studies.[27] Obviously, in neuropathic conditions, local somatopic changes occur at spinal levels; the α_2-δ_1 subunit of the voltage-gated calcium channel is known to be significantly upregulated in the dorsal horn of the spinal cord on the ipsilateral side of the nerve injury only, as compared with the contralateral (nonligated) side.[4] Peripheral neuropathy therefore alters central modulation of pain through altered descending controls with global changes as well as highly specific pharmacological changes such as those that we describe.

Conditioned pain modulation is the term used to describe a human paradigm in which a conditioning stimulus is used to affect a distant test stimulus.[34] Conditioned pain modulation paradigms can be used to assess the efficacy of the DNIC system as a surrogate measure of descending inhibition and are diminished in many patients with chronic pain who are suffering from conditions including osteoarthritis, fibromyalgia, and diabetic neuropathy.[1,19,36] Meanwhile, a weak CPM is predictive of those initially pain-free patients who are more likely to develop postsurgical persistent pain.[35] Animals who received sham surgery had an identical DNIC to control unoperated animals. The lack of any postsurgical pain phenotype in this group with the maintenance of DNIC translates perfectly to patient data. There is evidence that in a population of patients without pain, only 8% of individuals exhibit little or no CPM.[22] Multiple peripheral and

central mechanisms will contribute to the variability of the pain experience for neuropathic pain patients,[35] and yet both a reduction[36] and a complete loss of DNIC (or CPM) are observed in patients with diabetic polyneuropathy.[24] In the SNL animals, DNIC was lost for the majority of neurons studied, both within the damaged nerve territory and contralaterally. An increased activation of DNIC in rats with chronic constriction injury of the sciatic nerve, whilst maintaining normal DNIC elsewhere, has been reported.[10] These findings are in contrast to our results where DNIC was completely abolished in the SNL model. They also contrast with a wealth of evidence supporting the idea that there is a loss of descending inhibition in varied human chronic pain states. These differences could relate to different models of neuropathy with the SNL mirroring the human data; 2 closely related strains of rat have different incidences of neuropathic pain behaviours even after SNL injury, proposed to relate to differences in descending modulation including noradrenergic systems.[11] Patients with neuropathic pain syndromes are heterogeneous, presenting with a variety of sensory symptoms and pain qualities.[3] It is possible that variations in CPM reflect the heterogeneity of patients. Thus, between-study differences in DNIC might be related to differences in the strain of rats studied and/or the model of neuropathy used as well as other variables. It seems likely that there may be specific pathologies or sensory phenotypes linked to reductions in DNIC and CPM.

Duloxetine, a 5-HT–norepinephrine reuptake inhibitor (SNRI), enhances descending inhibitory pain controls. It was hypothesized that in patients with less efficient CPM, duloxetine would be more beneficial as a pain-relieving agent. Indeed, low or absent CPM predicts duloxetine efficacy in patients with diabetic neuropathy. In contrast, temporal summation (a measure of enhanced sensitization) does not.[36] These findings corroborate that the site and mechanisms underlying temporal summation and DNIC are distinct with the former being a spinal event[23] and the latter being an independent descending inhibitory control acting alongside intrinsic spinal events. They also support the notion that SNRIs do not directly target neuronal sensitization. With duloxetine, it may be that the 5-HT actions do not necessarily aid pain control given the facilitatory effects of the 5-HT_3 receptor. 5-HT is involved in DNIC.[12] However, 5-HT_7 receptors are involved in descending inhibition, and the relative actions of altered 5-HT levels on the multiple receptors in vivo are not easily predictable. Neither is the relative norepinephrine/5-HT uptake block.[13] Overall, norepinephrine acting through α_2 ARs is a more clear antinociceptive target.

The triggers for the altered descending controls in SNL are not fully understood. In particular, it is unclear how inhibitions are lost and facilitations enhanced. It could be a compensation by central nervous system areas for the partial loss of afferent input; it is known that there is a loss of spinal neuronal windup and also a disruption in descending controls after ablation of spinal lamina I/III NK1 projection neurons using a substance P and saporin conjugate (SP-SAP). Not only are α_2 AR controls and 5-HT_3 receptor-mediated facilitations altered but also DNICs are markedly reduced by this interference with spinal processing. These neurons project to the parabrachial area, which in turn connects to areas such as the RVM, LC, and SRD, and so changes in peripheral nerve inputs in neuropathy onto these neurons could in turn alter the output of the descending systems.[29] Indeed, dorsal root sections switched some descending controls from LC and RVM, triggered by electrical stimulation from inhibition to excitation.[15]

Diffuse noxious inhibitory controls and CPM translate in both directions in terms of their functional effects and pharmacological substrates. The functionality and pharmacology of DNIC/CPM are thus remarkably similar, supporting their translational value. Balancing excitations and inhibitions with drugs acting on monoamine systems may be of benefit not only in restoring normal descending inhibitory balance but also conceivably in prevention of persistent postsurgical and other pains because impaired CPM is a predictor. These principles may also apply to the control of many pains other than neuropathy because altered CPM has been described in many patient groups.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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