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Neuroendocrine Stress Axis-Dependence of Duloxetine Analgesia (Anti-Hyperalgesia) in Chemotherapy-Induced Peripheral Neuropathy

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Duloxetine, a serotonin and norepinephrine reuptake inhibitor, is the best-established treatment for painful chemotherapy-induced peripheral neuropathy (CIPN). While it is only effective in little more than half of patients, our ability to predict patient response remains incompletely understood. Given that stress exacerbates CIPN, and that the therapeutic effect of duloxetine is thought to be mediated, at least in part, via its effects on adrenergic mechanisms, we evaluated the contribution of neuroendocrine stress axes, sympathoadrenal and hypothalamic-pituitary-adrenal, to the effect of duloxetine in preclinical models of oxaliplatin- and paclitaxel-induced CIPN. Systemic administration of duloxetine, which alone had no effect on nociceptive threshold, both prevented and reversed mechanical hyperalgesia associated with oxaliplatin- and paclitaxel-CIPN. It more robustly attenuated oxaliplatin CIPN in male rats, while it was more effective for paclitaxel CIPN in females. Gonadectomy attenuated these sex differences in the effect of duloxetine. To assess the role of neuroendocrine stress axes in the effect of duloxetine on CIPN, rats of both sexes were submitted to adrenalectomy combined with fixed level replacement of corticosterone and epinephrine. While CIPN, in these rats, was of similar magnitude to that observed in adrenal-intact animals, rats of neither sex responded to duloxetine. Furthermore, duloxetine blunted an increase in corticosterone induced by oxaliplatin, and prevented the exacerbation of CIPN by sound stress. Our results demonstrate a role of neuroendocrine stress axes in duloxetine analgesia (anti-hyperalgesia) for the treatment of CIPN.

Key words: **CIPN; oxaliplatin; paclitaxel; pain; sex differences; stress**

Significance Statement

Painful chemotherapy-induced peripheral neuropathy (CIPN) is a debilitating dose-dependent and therapy-limiting side effect of many of the cytostatic drugs used to treat cancer (Argyriou et al., 2010; Marmiroli et al., 2017). Duloxetine is the only treatment for CIPN currently recommended by the American Society of Clinical Oncology (Hershman et al., 2014). In the present study, focused on elucidating mechanisms mediating the response of oxaliplatin- and paclitaxel-induced painful peripheral neuropathy to duloxetine, we demonstrate a major contribution to its effect of neuroendocrine stress axis function. These findings, which parallel the clinical observation that stress may impact response of CIPN to duloxetine (Taylor et al., 2007), open new approaches to the treatment of CIPN and other stress-associated pain syndromes.

Introduction

Peripheral neuropathy is a major dose-limiting side effect of many first-line drugs used to treat cancer (Hershman et al., 2014; Marmiroli et al., 2017; Loprinzi et al., 2020). Several of these chemotherapies, including taxanes and platinum-based compounds (Staff et al., 2017), produce peripheral neuropathy as a side effect in 38%-90% of patients (Petrovchich et al., 2019) that can last for months or even years after stopping therapy (Staff et al., 2017). Painful chemotherapy-induced peripheral neuropathy (CIPN) greatly impacts quality of life in oncology patients and can lead to reduction or even discontinuation of therapy, thereby

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compromising effective cancer treatment (Wright et al., 2020). While the only drug currently recommended by the American Society of Clinical Oncology for the treatment of CIPN is duloxetine (Hershman et al., 2014; Loprinzi et al., 2020), it is only effective in reducing CIPN pain in ~60% of patients (Smith et al., 2013). Determining factors that contribute to duloxetine efficacy in CIPN is hampered by our incomplete understanding of the mechanism by which it relieves neuropathic pain.

We and others have shown that stress, which plays a substantial role in the symptom burden of oncology patients (Miaskowski et al., 2018b; Mazor et al., 2019; Jakovljevic et al., 2021), is a risk factor for developing CIPN (Miaskowski et al., 2018a), and that CIPN pain is worse in patients with greater levels of stress (Kober et al., 2018). Validating this link, we have demonstrated a contribution of the sympathoadrenal and hypothalamic-pituitary-adrenal (HPA) neuroendocrine stress axes in preclinical models of painful oxaliplatin and paclitaxel CIPN (Ferrari et al., 2020; Staurengo-Ferrari et al., 2021). Since stress phenotype may influence response of duloxetine in patients with CIPN pain (Smith et al., 2017), we tested the hypothesis that duloxetine targets neuroendocrine stress axes to alleviate CIPN pain. Of note in this regard, duloxetine is used extensively in the management of pain syndromes that are induced or exacerbated by stress, for example, fibromyalgia (Rodrigues-Amorim et al., 2020), chronic musculoskeletal pain (Rodrigues-Amorim et al., 2020; Scherf-Clavel et al., 2020), and migraine (Taylor et al., 2007; Kisler et al., 2019).

Another predictor of the response of CIPN to duloxetine is related to the chemotherapeutic agent administered, with patients receiving oxaliplatin more likely to experience a benefit from duloxetine than patients treated with paclitaxel (Smith et al., 2013). Thus, duloxetine's mechanism of action may be tied, at least in part, to specific mechanisms of chemotherapy-induced neurotoxicity. In this study, we used established preclinical models of CIPN produced by two commonly used neurotoxic chemotherapeutic agents, oxaliplatin (Joseph et al., 2008; Alvarez et al., 2011; Staurengo-Ferrari et al., 2021) and paclitaxel (Dina et al., 2001; Alvarez et al., 2011; Ferrari et al., 2020), to evaluate the contribution of neuroendocrine stress axes in the therapeutic response of CIPN to duloxetine. Additionally, because of the marked sex differences on the role of the neuroendocrine stress axes in CIPN (Ferrari et al., 2020; Staurengo-Ferrari et al., 2021), we also evaluated for sex differences associated with the response of CIPN to duloxetine.

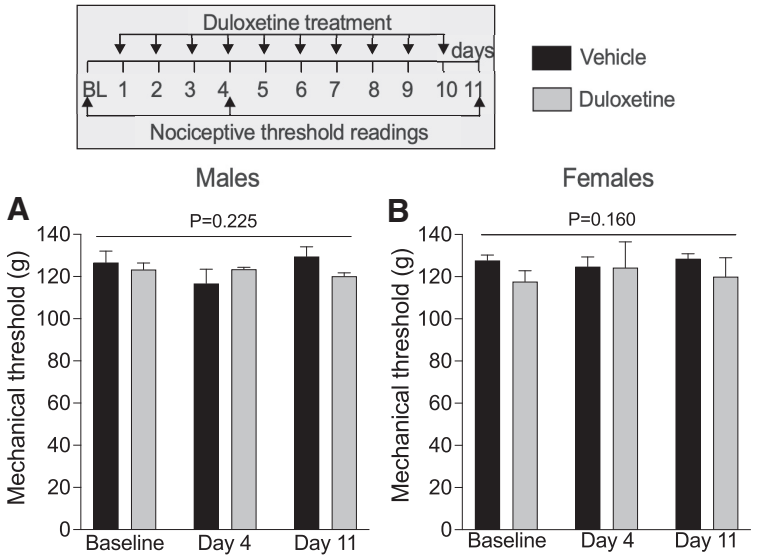

Figure 1. Effect of duloxetine on nociceptive threshold. Groups of male and female rats were treated intraperitoneally with duloxetine (10 mg/kg, i.p.) or its vehicle (2% of DMSO plus saline, i.p.), for either 3 or 10 consecutive days. Before treatment (baseline) and 24 h after the last administration of duloxetine (after 3 or 10 d of treatment), mechanical nociceptive threshold was evaluated using the Randall–Selitto paw-withdrawal test. ***A***, Males. Administration of duloxetine did not affect mechanical nociceptive threshold in male rats treated with duloxetine for either 3 or 10 consecutive days compared with the vehicle-treated control group (two-way ANOVA duloxetine vs vehicle: duloxetine × time interaction, $F_{(2,20)} = 1.608$, not significant). Data are mean ± SEM; $n = 6$/group. ***B***, Females. Administration of duloxetine also did not affect mechanical nociceptive threshold in females treated with duloxetine for 3 or 10 consecutive days compared with the vehicle control group (two-way ANOVA, duloxetine vs vehicle: duloxetine × time interaction, $F_{(2,20)} = 2.012$, not significant). Data are mean ± SEM; $n = 6$/group.

Materials and Methods

Animals

Experiments were performed on male and female Sprague Dawley rats (220-400 g, purchased from Charles River Laboratories). Experimental animals were housed same sex, 3 per cage, under a 12 h light/dark cycle (lights on 07:00), in a temperature- and humidity-controlled animal care facility at the University of California, San Francisco. Food and water were available *ad libitum*. Experimental protocols were approved by the University of California, San Francisco Institutional Animal Care and Use Committee and adhered to the guidelines of the American Association of Laboratory Animal Care, the National Institutes of Health, and the Committee for Research and Ethical Issues of the International Association for the Study of Pain, for the use of animals in research.

Nociceptive threshold testing

Mechanical nociceptive threshold was measured using a Ugo Basile Analgesy-Meter (Randall–Selitto paw-withdrawal test; Stoelting), which applies a linearly increasing mechanical force to the dorsum of the rat's hind paw, as previously described (Randall and Selitto, 1957; Taiwo et al., 1989). Rats were placed in cylindrical acrylic restrainers designed to minimize restraint stress and allow hind leg extension from lateral ports, for the assessment of nociceptive threshold, to be measured on the dorsum of the hind paw. To acclimatize rats to the testing procedure, they were placed in a restrainer for 1 h before experimental manipulations. Nociceptive threshold was defined as the force, in grams, at which a rat withdrew its paw. Baseline nociceptive threshold was defined as the mean of three readings taken before injection of test agents. To minimize experimenter bias, individuals conducting behavioral experiments were blinded to experimental interventions.

Drugs

The following drugs were used in this study: oxaliplatin, paclitaxel, epinephrine, and corticosterone, purchased from Sigma-Aldrich; and, duloxetine hydrochloride, purchased from TCI America.

Duloxetine treatment. Duloxetine was dissolved in 0.9% NaCl containing 2% DMSO, for intraperitoneal injection at a dose of 10 or 20 mg/kg; the injectate concentration of duloxetine was adjusted to 1 mg/ml. To evaluate its analgesic effect on oxaliplatin or paclitaxel CIPN, long-term regimens of duloxetine administration were used. This treatment protocol was chosen to reflect its clinical use (Smith et al., 2013; Stockstill et al., 2020). To evaluate whether duloxetine could delay or prevent the development of oxaliplatin- or paclitaxel-induced hyperalgesia, male and female rats were treated with duloxetine in a prevention protocol. In this protocol, treatment with duloxetine was started 3 d before administration of oxaliplatin (2 mg/kg, i.v.) or the first (of four)

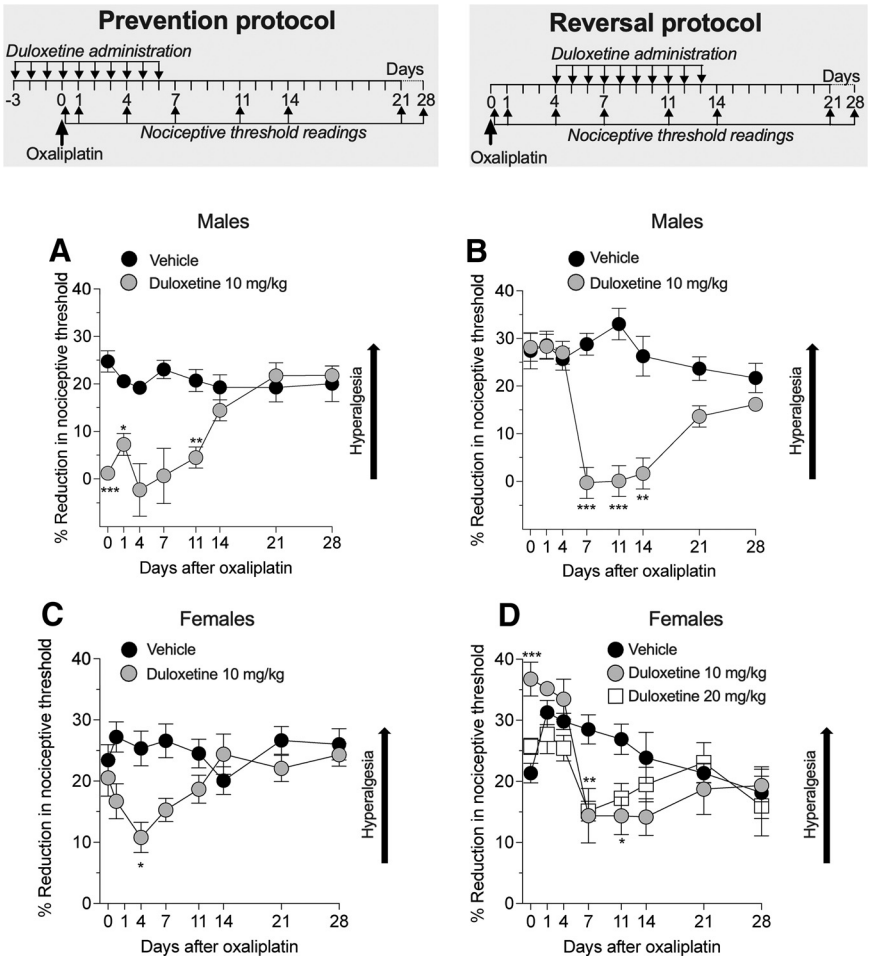

Figure 2. Sex difference in duloxetine anti-hyperalgesia for oxaliplatin CIPN. Both male and female rats were treated intraperitoneally with duloxetine or its vehicle, for 10 consecutive days, in prevention and reversal protocols. Prevention protocol: oxaliplatin (2 mg/kg, i.v.) was administered ~24 hours after the third daily injection of duloxetine or vehicle (day 0). Reversal protocol: treatment with duloxetine or vehicle started 4 days after administration of oxaliplatin (2 mg/kg, i.v.). In both protocols, mechanical nociceptive threshold was evaluated before oxaliplatin administration and again 30 minutes and 1, 4, 7, 11, 14, 21, and 28 d later. Males: ***A***, The magnitude of oxaliplatin-induced hyperalgesia was markedly attenuated in male rats treated with duloxetine (10 mg/kg, i.p.) in the prevention protocol, 30 min and on days 1, 4, 7, and 11 after oxaliplatin administration. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(7,70)} = 9.06$, $p < 0.0001$; duloxetine treatment, $F_{(1,10)} = 23.99$, $p = 0.0006$; Bonferroni's multiple *post hoc* comparisons test: ***$p < 0.0001$, **$p = 0.004$, *$p = 0.018$: duloxetine, oxaliplatin group versus vehicle, oxaliplatin group (n = 6/group). ***B***, The magnitude of oxaliplatin-induced hyperalgesia was also markedly attenuated in male rats treated with duloxetine (10 mg/kg, i.p.) in the reversal protocol, on days 7, 11, and 14 after oxaliplatin administration. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(7,70)} = 15.25$, $p < 0.0001$; duloxetine treatment, $F_{(1,10)} = 29.10$, $p = 0.0003$; Bonferroni's multiple *post hoc* comparisons test: ***$p = 0.003$, **$p = 0.087$ (n = 6/group). Females: ***C***, The magnitude of oxaliplatin-induced hyperalgesia was modestly attenuated in female rats treated with duloxetine, in the prevention protocol, measured on days 1, 4, and 7 after oxaliplatin administration. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(7,70)} = 5.54$, $p < 0.0001$; duloxetine treatment, $F_{(1,10)} = 5.047$, $p = 0.0485$; Bonferroni's multiple *post hoc* comparisons test: **$p = 0.026$ (n = 6/group). ***D***, The magnitude of oxaliplatin-induced hyperalgesia was also modestly attenuated by duloxetine (10 or 20 mg/kg, i.p.) in the reversal protocol, in females, on days 7 and 11. No statistical differences between the two duloxetine doses were detected. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine treatment, $F_{(14,105)} = 3.44$, $p = 0.0001$; duloxetine treatment, $F_{(2,15)} = 2.80$, $p = 0.092$; Bonferroni's multiple *post hoc* comparisons test: ***$p = 0.0041$ (vehicle vs 10 mg/kg duloxetine), **$p = 0.0039$ (vehicle vs 20 mg/kg duloxetine), *$p = 0.0032$ (vehicle vs 10 mg/kg duloxetine) (n = 6/group).

doses of paclitaxel (1 mg/kg, i.p.). To evaluate whether duloxetine could attenuate established oxaliplatin- or paclitaxel-induced hyperalgesia, a reversal protocol was used. In this protocol, duloxetine treatment was started 4 d after administration of oxaliplatin, or 4 d after the last dose of paclitaxel, at which times robust hyperalgesia was present. In sound stress experiments, duloxetine treatment was started 11 d after sound stress, which corresponds to 3 d before chemotherapy (day 14); we have previously shown that the maximal effect on nociceptive responses occurs 14 d after sound stress (Khasar et al., 2005, 2008). Rats received duloxetine daily for 10 d in both prevention and reversal protocols. Duloxetine dose was chosen based on previous results that evaluated the effect of duloxetine in models of inflammatory and neuropathic pain (Kim et al., 2017; Minami et al., 2018; Kajiwara et al., 2020; Stockstill et al., 2020).

Preclinical model of oxaliplatin-induced neuropathic pain (CIPN). A single dose of oxaliplatin (2 mg/kg, i.v.) or, in control rats its vehicle (saline), was administered (Joseph et al., 2008). This is a well-described and highly reproducible model in which both phases of oxaliplatin CIPN are observed: an early phase, marked by acute onset, which lasts several hours to a few days (Grothey and Goldberg, 2004; Saif and Reardon, 2005), followed by a mechanistically distinct late phase (Cersosimo, 2005), that persists in this model for the 28 d testing period (Joseph et al., 2008; Staurengo-Ferrari et al., 2021).

Preclinical model of paclitaxel-induced neuropathic pain (CIPN). Paclitaxel was prepared in a mixture of absolute ethanol and polyethoxylated castor oil (Cremophor EL; 1:1; Sigma-Aldrich) and further diluted in saline, to a concentration of 1 mg/ml, immediately before use (Ferrari et al., 2018, 2020). Paclitaxel was injected intraperitoneally every other day for a total of 4 doses (1 mg/kg × 4); control animals received an equivalent volume of vehicle with proportional amounts of Cremophor EL and 95% ethanol diluted in saline (Ferrari et al., 2018, 2020).

Surgical procedures

For all surgical procedures, animals were anesthetized with isoflurane (3% in oxygen). Incision areas were shaved, and skin swabbed with povidone iodine solution, followed by 70% ethanol; and to provide perioperative analgesia, rats were injected with meloxicam (~5 mg/kg, s.c.) and bupivacaine (~5-8 mg/kg, infiltrated intradermally at the incision site). During surgery, rats were placed on a heating pad to maintain core body temperature at 37°C.

Gonadectomy. Male and female rats, gonadectomized at 3 weeks of age (i.e., prepubertal), were used for behavioral experiments, 4 weeks later (i.e., as adults) (Green et al., 2001). Ovaries were accessed through bilateral flank wall abdominal incisions (Bonet et al., 2021). The ovarian bundles were located, tied off with 5–0 silk suture (Ethicon, Johnson & Johnson), and the ovaries excised. Fascia and skin were then closed with 5–0 silk suture.

To perform bilateral orchiectomy, a single cutaneous incision was made through the scrotal skin and underlying tunica, to expose the testes. Testicular vascular bundles were located, tied off with 5–0 silk suture, and the testes excised. The cutaneous incision was then closed with 5–0 silk suture.

Adrenalectomy. Adrenalectomies were performed on 220-240 g adult rats. Two flank wall abdominal incisions were made, and adrenal glands visualized and excised (Green et al., 1995); 5-0 silk suture was used to separately close abdominal wall and skin incisions. Control animals

underwent sham adrenalectomy, in which the adrenal glands were visualized but not excised.

Stress hormone replacement

Stress hormones, epinephrine and corticosterone, were administered to produce plasma levels observed in mildly stressed rats. Epinephrine was delivered by osmotic minipumps at a rate of 5.4 μg/0.25 μl/h, which produces plasma levels of 720 pg/ml in rats (Strausbaugh et al., 2003). Corticosterone was administered by a slow-release pellet, 100 mg of fused corticosterone, which also produces plasma corticosterone in the range induced by mild chronic stress (Meyer et al., 1979; Duclos et al., 2004). Pumps and slow-release pellets, implanted subcutaneously in the interscapular region, delivered stress hormones over a period of 28 d. Oxaliplatin (2 mg/kg, i.v.) or first dose of paclitaxel (1 mg/kg × 4, i.p.) was injected 1 d after rats were submitted to adrenalectomy and stress hormone implants.

Unpredictable sound stress

To induce sustained increase in stress hormone levels, we used a well-established stress model, unpredictable sound stress (Singh et al., 1990; Strausbaugh et al., 2003). Groups of 3 adult rats were placed in a 30 × 40 × 24 cm wire mesh cage, 25 cm from a loudspeaker, inside a 56 × 56 × 72 cm sound-insulated box. Sound pulses were emitted as pure tones, at three frequencies (11, 15, and 19 kHz) with amplitudes varying from 20 to 110 dB, independently for each frequency. The sound stress protocol was initiated after placing rats in the wire mesh cage and terminated 30 min later. Over the 30 min period, a 5 or 10 s tone was presented every minute, at random times. Exposure to unpredictable sound stress occurred on days 1, 3, and 4. Sham stressed animals were placed in the chamber used for sound stress for 30 min at the same time points, but without exposure to the sound stimulus. After sound stress or sham treatment, rats were returned to their home cages. Fourteen days after the last exposure to sound stress or sham sound stress, over which time neuroplastic changes induced by sound stress are established (Khasar et al., 2008; Staurengo-Ferrari et al., 2021), animals were used for behavioral experiments.

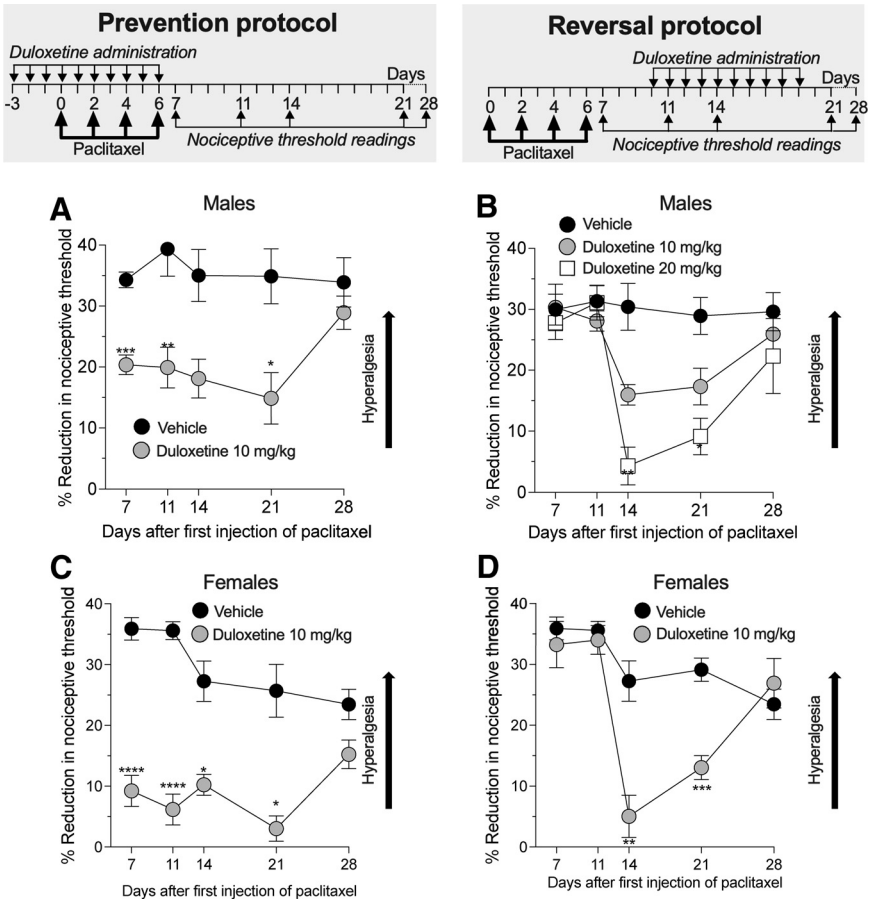

Figure 3. Sex difference in the anti-hyperalgesic effect of duloxetine in paclitaxel CIPN. Male and female rats were treated with duloxetine or its vehicle, intraperitoneally, for 10 consecutive days, in prevention and reversal protocols. Prevention protocol: starting ~24 h after the third dose of duloxetine or vehicle, paclitaxel (1 mg/kg, i.p.) was administered every other day for a total of 4 injections, on days 0, 2, 4, and 6 (1 mg/kg × 4, i.p.). Reversal protocol: treatment with duloxetine or vehicle started 4 days after the last injection of paclitaxel (1 mg/kg × 4, i.p.). Males: ***A***, The magnitude of paclitaxel-induced hyperalgesia was modestly attenuated in male rats treated with duloxetine (10 mg/kg, i.p.) in the prevention protocol, on days 7, 14, and 21. Data are mean ± SEM. Two-way repeated-measures ANOVA, interaction, $F_{(4,40)} = 2.037$, $p = 0.1075$; duloxetine treatment, $F_{(1,10)} = 21.82$, $p = 0.0009$; Bonferroni's multiple *post hoc* comparisons test: ***$p = 0.0003$, **$p = 0.0326$, *$p = 0.0448$ ($n = 6$/group). ***B***, Paclitaxel-induced hyperalgesia was also transiently attenuated in males treated with duloxetine (10 mg/kg, i.p.) in the reversal protocol, measured on day 14. In contrast, in this protocol, duloxetine (20 mg/kg, i.p.) more strongly attenuated hyperalgesia induced by paclitaxel, in males, on days 11, 14, and 21. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(8,60)} = 3.96$, $p = 0.0008$; duloxetine treatment, $F_{(2,15)} = 7.95$, $p = 0.0044$; Bonferroni's multiple *post hoc* comparisons test: **$p = 0.0063$ (vehicle vs 20 mg/kg duloxetine), *$p = 0.0138$ (vehicle vs 20 mg/kg duloxetine) ($n = 6$/group). No statistical differences between the effect of the two duloxetine doses were detected. Females: ***C***, Duloxetine markedly attenuated paclitaxel-induced hyperalgesia in females, on days 7, 11, 14, and 21, in the prevention protocol. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(4,40)} = 5.392$, $p = 0.0014$; duloxetine treatment, $F_{(1,10)} = 145.8$, $p < 0.0001$; Bonferroni's multiple *post hoc* comparisons test: ****$p < 0.0001$, *$p = 0.01$ ($n = 6$/group). ***D***, Duloxetine markedly, but transiently, attenuated paclitaxel-induced hyperalgesia in females, in the reversal protocol, on days 14 and 21. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(4,40)} = 10.49$, $p < 0.0001$; duloxetine treatment, $F_{(1,10)} = 8.88$, $p = 0.0138$; Bonferroni's multiple *post hoc* comparisons test: ***$p = 0.0008$, **$p = 0.0047$ ($n = 6$/group).

Measurement of plasma corticosterone

Afternoon (PM, 16:00-17:00) and morning (AM, 08:30-09:30) blood samples were collected 1 d before administration of oxaliplatin or duloxetine (prevention protocol) and on days 1 and 7 after oxaliplatin, in males. To minimize stress of blood collection, rats were briefly anesthetized with 3% isoflurane. Peripheral blood samples were collected from a tail vein, and put in heparinized tubes, centrifuged, and plasma removed; plasma samples were stored at −80°C until analysis. Plasma corticosterone levels were assayed using an ELISA kit (Enzo Biochem), with plates read on a Wallac 1420 Victor2 Microplate Reader (PerkinElmer). Plasma concentration of corticosterone was calculated by interpolation from standard curves using Prism 9 software (GraphPad).

Data analysis

For the comparison of mechanical nociceptive thresholds, before and after duloxetine treatment, results are presented as absolute values in grams. To assess the effect of duloxetine on oxaliplatin- and paclitaxel-induced hyperalgesia, results are expressed as percentage change from baseline paw withdrawal threshold. For time course studies, repeated-measures two-way ANOVA followed by Bonferroni *post hoc* test were used. To test the hypothesis that oxaliplatin increases corticosterone and that this increase is attenuated by duloxetine, we use one-tailed Student's *t* test to compare plasma corticosterone levels before and after oxaliplatin administration (with and without duloxetine pretreatment). The significance level for all analyses was set at $p < 0.05$. To compare groups, data, presented as mean ± SEM, were analyzed using Prism 9 software.

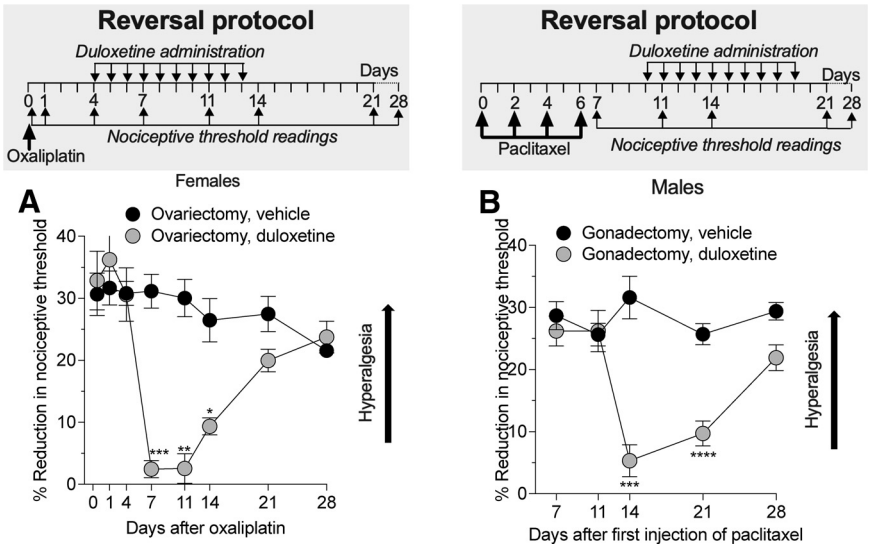

Figure 4. Role of gonadal hormones in response of CIPN to duloxetine. Females: Female rats were submitted to ovariectomy. Four weeks later, oxaliplatin was administered (2 mg/kg, i.v.). Four days after oxaliplatin, rats were treated with duloxetine (reversal protocol) or its vehicle (DMSO 2% plus saline). Mechanical nociceptive threshold was evaluated before oxaliplatin administration and again at 30 minutes, and on days 1, 4, 7, 11, 14, 21, and 28 after intravenous administration of oxaliplatin. ***A***, The magnitude of oxaliplatin-induced hyperalgesia was markedly attenuated by duloxetine in gonadectomized females, in the reversal protocol, on days 7, 14, and 21. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(7,70)} = 11.18$, $p < 0.0001$; duloxetine treatment, $F_{(1,10)} = 22.04$, $p = 0.0008$; Bonferroni's multiple *post hoc* comparisons test: ***$p = 0.002$, **$p = 0.003$, *$p = 0.025$ ($n = 6$/group). Furthermore, the attenuation of hyperalgesia by duloxetine was not significantly different between gonadectomized females and gonadal intact males (two-way repeated-measures ANOVA, days 7-14, female ovariectomy, oxaliplatin, duloxetine group vs male, oxaliplatin, duloxetine group, $F_{(1,10)} = 4.45$, not significant). Males: Four weeks after gonadectomy, in males, paclitaxel was administered (1 mg/kg × 4, i.p.). Five days after the last injection of paclitaxel (~day 11), rats were treated with duloxetine (10 mg/kg, i.p.) (reversal protocol) or its vehicle (DMSO 2% in saline). Mechanical nociceptive threshold was evaluated before the first dose of paclitaxel and again 7, 14, 21, and 28 d after intravenous administration of oxaliplatin. ***B***, The magnitude of oxaliplatin-induced hyperalgesia was fully attenuated in gonadectomized males treated with duloxetine, in the reversal protocol, on days 14 and 21. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(4,40)} = 9.213$, $p < 0.0001$; duloxetine treatment, $F_{(1,10)} = 97.72$, $p < 0.0001$; Bonferroni's multiple *post hoc* comparisons test: ****$p = 0.0007$, **$p = 0.0008$ ($n = 6$/group).

Results

Effect of duloxetine on nociceptive threshold

Since it is generally presumed that duloxetine produces analgesia, at least in part, by action in the CNS, to enhance descending antinociceptive controls (Kremer et al., 2018), we first evaluated whether systemic administration of duloxetine (10 mg/kg, i.p.) increases nociceptive threshold in control male (Fig. 1*A*) and female (Fig. 1*B*) rats. Two duloxetine treatment regimens were used: three daily doses of duloxetine (the doses just before administration of chemotherapy); and 10 daily doses of duloxetine (equivalent to the number of doses administered to CIPN animals). Duloxetine did not affect nociceptive threshold of animals treated for either 3 or 10 d, in either sex (Fig. 1*A*,*B*), compared with vehicle-treated groups. Since no change in the nociceptive threshold was observed, we use the term anti-hyperalgesia to describe duloxetine effects in models of CIPN, which is characterized by mechanical hyperalgesia.

Response of oxaliplatin CIPN to duloxetine

Two treatment protocols were used to evaluate the effect of duloxetine on oxaliplatin CIPN: (1) a prevention protocol, to determine whether duloxetine would delay the onset or attenuate the magnitude of oxaliplatin-induced hyperalgesia; and (2) a reversal protocol, to determine whether duloxetine can reverse established oxaliplatin-induced hyperalgesia. Since the clinical efficacy of duloxetine in the treatment of CIPN is based on chronic administration (Smith et al., 2013), rats received duloxetine for 10 consecutive days, in both treatment protocols.

In the prevention protocol, starting duloxetine 3 d before administration of oxaliplatin (2 mg/kg, i.v., day 0), there was a marked attenuated oxaliplatin-induced hyperalgesia in male rats (Fig. 2*A*). Although duloxetine treatment was suspended on day 6 after oxaliplatin, its anti-hyperalgesic effect persisted for an additional 5 d, after which the magnitude of oxaliplatin-induced hyperalgesia was similar to that seen in the vehicle-treated group (Fig. 2*B*). In the reversal protocol, when duloxetine treatment was started on day 4 after oxaliplatin administration, it reversed oxaliplatin-induced hyperalgesia in male rats, measured at days 7, 11, and 14 (Fig. 2*C*), suggesting that the effect of duloxetine is similar in its effect on both the initiation and maintenance of oxaliplatin CIPN, in male rats. These effects were observed when male rats were treated at a dose of 10 mg/kg, intraperitoneally. In females, treated with this dose, duloxetine only modestly, but significantly, attenuated oxaliplatin-induced hyperalgesia, in both prevention (Fig. 2*C*) and reversal (Fig. 2*D*) treatment protocols. A higher dose of duloxetine (20 mg/kg, i.p.), in the reversal protocol (Fig. 2*D*), in females, did not produce any greater attenuation of oxaliplatin-induced hyperalgesia.

Response of paclitaxel CIPN to duloxetine

To assess its effect on paclitaxel CIPN, duloxetine was also administered in both prevention and reversal protocols, daily for 10 d. In the prevention protocol (Fig. 3*A*,*C*), duloxetine was started 3 d before the first injection of paclitaxel (1 mg/kg, i.p.) and treatment continued for 1 week, which corresponds to the last dose of paclitaxel. In the prevention protocol, duloxetine (10 mg/kg, i.p.) produced only a small, albeit significant, attenuation of paclitaxel-induced hyperalgesia in male rats (Fig. 3*A*), markedly contrasting with the effect of duloxetine pretreatment in oxaliplatin CIPN in male rats (Fig. 2*A*). In contrast, in females, duloxetine pretreatment produced a very robust anti-hyperalgesia (Fig. 3*C*). Similar sex differences in the effect of duloxetine were observed for paclitaxel CIPN in the reversal protocol (Fig. 3*B*,*D*), suggesting that, despite the shared ability of paclitaxel (Dina et al., 2001) and oxaliplatin (Joseph et al., 2008) to induce nociceptor sensitization, the response to duloxetine in CIPN relies on different, sex-dependent, mechanisms. Administration of a higher dose (20 mg/kg) of duloxetine in males, in the reversal protocol, produced a greater attenuation of paclitaxel-induced hyperalgesia (Fig. 3*A*,*B*), compatible with a shift to the right in the dose dependence for duloxetine anti-hyperalgesia. For both doses, when duloxetine treatment was suspended, the hyperalgesia induced by paclitaxel gradually returned to a level observed in the vehicle-treated group.

Gonadal dependence of sex differences in response to duloxetine

Based on the sex differences in the response to duloxetine, we evaluated whether it is gonad hormone-dependent. Because the magnitude of the effect of duloxetine (10 mg/kg, i.p.) on oxaliplatin CIPN was greater in gonad intact males (Fig. 2), females were submitted to ovariectomy to assess whether female sex hormones suppress the therapeutic effect of duloxetine (Fig. 4*A*). Ovariectomy had no effect on the hyperalgesia induced by oxaliplatin, and the effect of duloxetine in ovariectomized and intact males is not statistically different.

In contrast to the response observed in oxaliplatin CIPN, duloxetine more robustly attenuated paclitaxel CIPN in females. Therefore, males were submitted to gonadectomy to evaluate whether male sex hormones suppress the therapeutic effect of duloxetine in paclitaxel CIPN (Fig. 4*B*). Gonadectomized males show a more robust response to duloxetine (10 mg/kg) (Fig. 4*B*). Together, these observations support the suggestion that gonadal hormones modulate the sensitivity of oxaliplatin and paclitaxel CIPN to duloxetine.

Role of neuroendocrine stress axes in CIPN response to duloxetine

To evaluate the role of neuroendocrine stress axes to the effect of duloxetine on oxaliplatin- and paclitaxel-induced hyperalgesia (Fig. 5), male and female rats were adrenalectomized, and epinephrine-containing osmotic minipumps and corticosterone-fused pellets implanted in the interscapular region 1 d before administration of oxaliplatin or the first dose of paclitaxel. Four days after oxaliplatin administration or 4 d after the last administration of paclitaxel, duloxetine (10 mg/kg) was administered in the reversal protocol. Oxaliplatin produced hyperalgesia of similar magnitude to that observed in adrenal-intact rats, for both sexes (Fig. 5*A*, *B*). However, in stress hormone-replaced adrenalectomized male and female rats, duloxetine failed to significantly attenuate hyperalgesia produced by either oxaliplatin (Fig. 5*A*,*B*) or paclitaxel (Fig. 5*C*,*D*). We have previously shown that, in adrenalectomized rats without replacement corticosterone and epinephrine, CIPN by oxaliplatin (Staurengo-Ferrari et al., 2021) and by paclitaxel (Ferrari et al., 2020) is completely prevented. These findings support the suggestion that mitigation of CIPN pain by duloxetine is neuroendocrine stress axis-dependent (Muscatello et al., 2019).

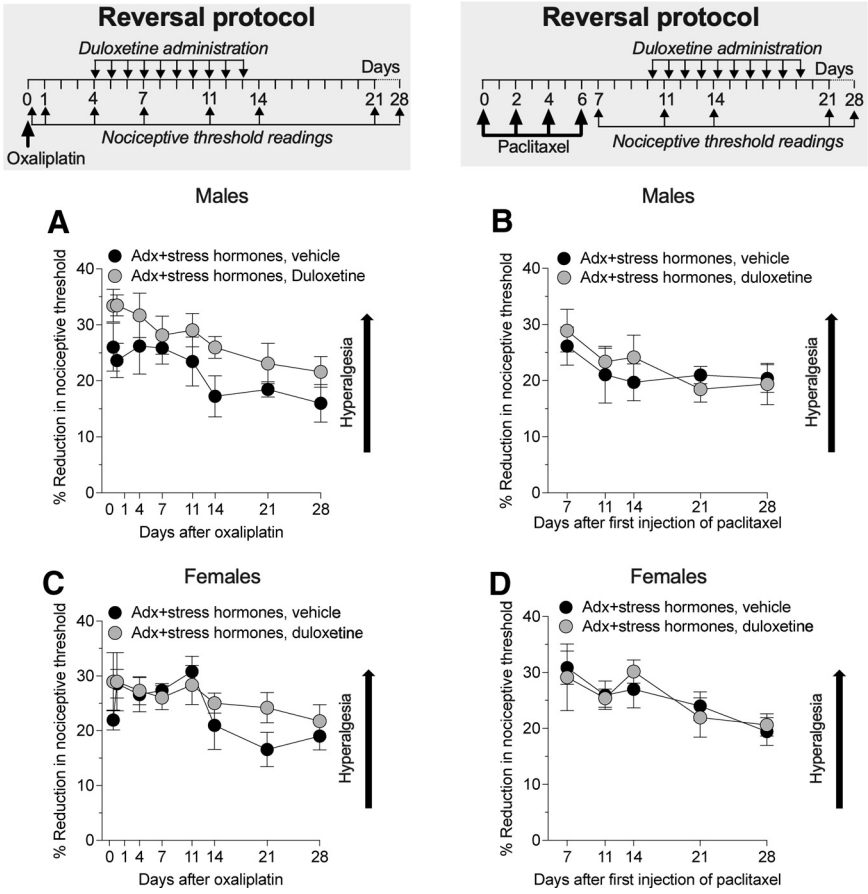

Figure 5. Role of neuroendocrine stress axes in the effect of duloxetine on oxaliplatin and paclitaxel CIPN. Adrenalectomized male and female rats were submitted to stable replacement of epinephrine (osmotic minipumps filled with 5.4 μg/0.25 μl/h of epinephrine) and corticosterone (100 mg in pellets). Twenty-four hours after surgical removal of the adrenal glands and implanting epinephrine-containing osmotic minipumps and corticosterone fused pellets, subcutaneously in the interscapular space, oxaliplatin (2 mg/kg, i.v.) or the first dose of paclitaxel (1 mg/kg × 4, i.p.) was administered. Four days after oxaliplatin or after the last dose of paclitaxel, rats were treated with duloxetine (reversal protocol) or its vehicle (DMSO 2% plus saline). Mechanical nociceptive threshold was evaluated before adrenalectomy and implant procedures, and again at 30 minutes, and on days 1, 4, 7, 11, 14, 21, and 28 after intravenous administration replacement. In paclitaxel CIPN, mechanical nociceptive threshold was evaluated before the adrenalectomy and implant procedures and again on days 7, 11, 14, 21, and 28 after the first dose of paclitaxel. Males: ***A***, Duloxetine did not attenuate oxaliplatin-induced hyperalgesia in adrenalectomized male rats implanted with stress hormone compared with vehicle treatment. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(7,70)} = 0.42$, $p = 0.887$; duloxetine treatment, $F_{(1,10)} = 3.7$, $p = 0.083$ ($n = 6$/group). ***B***, Duloxetine did not attenuate paclitaxel-induced hyperalgesia in adrenalectomized stress hormone-implanted male rats compared with vehicle treatment. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(4,40)} = 0.412$, $p = 0.799$; duloxetine treatment, $F_{(1,10)} = 0.225$, $p = 0.646$ ($n = 6$/group). Females: ***C***, Duloxetine did not attenuate oxaliplatin-induced hyperalgesia in adrenalectomized stress hormone-implanted female rats compared with vehicle treatment. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(7,70)} = 1.14$, $p = 0.348$; duloxetine treatment, $F_{(1,10)} = 0.534$, $p = 0.482$ ($n = 6$/group). ***D***, Duloxetine did not attenuate paclitaxel-induced hyperalgesia in adrenalectomized stress hormone-implanted female rats compared with vehicle treatment. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × duloxetine interaction, $F_{(4,40)} = 0.245$, $p = 0.919$; duloxetine treatment, $F_{(1,10)} = 0.00003$, $p = 0.996$ ($n = 6$/group).

Effect of duloxetine on stress-induced exacerbation of CIPN

To further test the hypothesis that mitigation of CIPN by duloxetine is because of its ability to attenuate stress axis function, we evaluated whether duloxetine could attenuate the exacerbation of CIPN induced by unpredictable sound stress, a protocol that stimulates the release of epinephrine and corticosterone from the adrenal gland (Strausbaugh et al., 2003; Khasar et al., 2009). Of note, because of the sex differences in the response of CIPN to duloxetine, specific sexes and chemotherapies were chosen to evaluate the effect of duloxetine in the setting of unpredictable sound stress.

As we previously observed (Ferrari et al., 2020; Staurengo-Ferrari et al., 2021), sound stress enhances oxaliplatin- and paclitaxel-induced hyperalgesia, without affecting nociceptive threshold, per se (Khasar et al., 2005). In oxaliplatin CIPN,

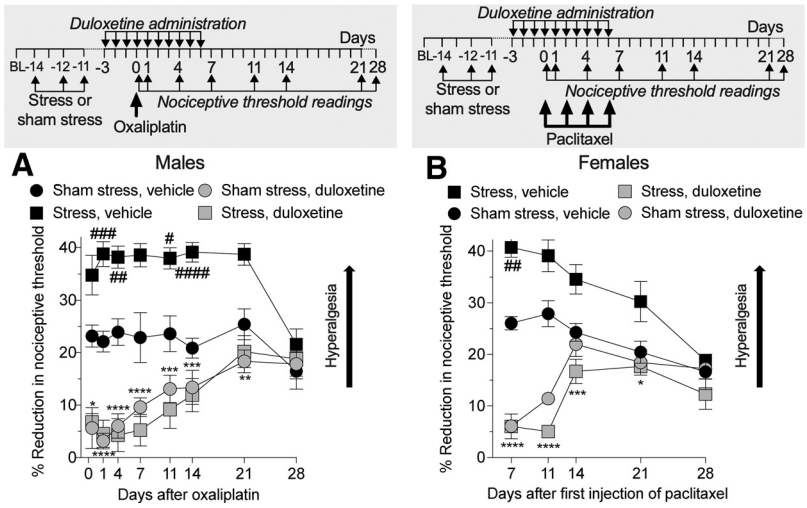

Figure 6. Duloxetine prevents sound stress-induced exacerbation of CIPN. Male and female rats were exposed to unpredictable sound stress for 3 days. Eleven days after the last exposure to sound stress, which corresponds to 3 d before chemotherapy administration (oxaliplatin or paclitaxel, day 0), duloxetine treatment (10 mg/kg, i.p.) was started. Animals were treated with duloxetine for 10 consecutive days. Males: ***A***, Duloxetine prevents the exacerbation of oxaliplatin hyperalgesia induced by sound stress, in males. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × treatment interaction, $F_{(21,140)} = 4.733$, $p < 0.0001$; treatment, $F_{(3,20)} = 55.98$, $p < 0.0001$ ($n = 6$/group). No significant differences between groups treated with duloxetine and submitted to sham or sound stress were detected. Bonferroni's multiple comparisons test, sham stress versus stress: $^{\#\#\#\#}p = 0.0003$, $^{\#\#\#}p = 0.002$, $^{\#\#}p = 0.009$, $^{\#}p = 0.04$; stress + vehicle versus stress + duloxetine: $^{****}p < 0.0001$, $^{***}p = 0.0008$ (day 11), $^{***}p = 0.0004$ (day 14), $^{**}p = 0.005$, $^{*}p = 0.017$. Females: ***B***, Duloxetine prevents the exacerbation of paclitaxel hyperalgesia by sound stress, in females. Data are mean ± SEM. Two-way repeated-measures ANOVA, time × treatment interaction, $F_{(12,80)} = 9.58$, $p < 0.0001$; treatment, $F_{(3,20)} = 62.48$, $p < 0.0001$ ($n = 6$/group). No significant differences between groups treated with duloxetine and submitted to sham or sound stress were detected. Bonferroni's multiple comparisons test, sham stress versus stress: $^{\#\#\#\#}p = 0.0003$, $^{\#\#\#}p = 0.002$, $^{\#\#}p = 0.009$, $^{\#}p = 0.04$; stress + vehicle versus stress + duloxetine: $^{****}p < 0.0001$, $^{***}p = 0.0008$ (day 11), $^{***}p = 0.0004$ (day 14), $^{**}p = 0.005$, $^{*}p = 0.017$. No significant differences between groups treated with duloxetine and submitted to sham or sound stress were detected.

duloxetine (10 mg/kg) prevented the enhancement of hyperalgesia induced by sound stress, in males, reaching similar magnitude of effect to that observed in sham stressed male rats treated with duloxetine (Fig. 6*A*). Albeit at lower magnitude, duloxetine anti-hyperalgesia continued to day 21, although duloxetine treatment had ended. Stress enhanced paclitaxel-induced hyperalgesia on days 7 and 11, and duloxetine prevented this enhancement (Fig. 6*B*).

Effect of duloxetine on corticosterone response to CIPN

To test the hypothesis that chemotherapy stimulates adrenal function, which can be attenuated by duloxetine, we assessed PM and AM plasma levels of corticosterone after administration of chemotherapy, including in rats treated with duloxetine. Measurement of both PM and AM corticosterone levels provides a more complete assessment of HPA axis function (Ockenfels et al., 1995; Adam, 2006; Adam et al., 2008; Adam and Kumari, 2009). These experiments were performed in male rats receiving oxaliplatin, chosen because males with oxaliplatin CIPN are particularly responsive to duloxetine. We measured PM and AM plasma corticosterone levels at three time points: (1) before administration of oxaliplatin (baseline); (2) the following day (acute response); and (3) 1 week after (late response) oxaliplatin. The same time points relative to oxaliplatin administration were used for rats that had been pretreated with duloxetine, starting 3 d before oxaliplatin administration and continued for a total of 10 d. While oxaliplatin induces an acute increase in AM corticosterone (1 d after its administration, 23.32 ± 9.59 pg/ml to 54.05 ± 10.01 pg/ml), there is a late response (1 week after oxaliplatin administration) in PM corticosterone (55.13 ± 9.22 pg/ml to 77.51 ± 12.99 pg/ml) (Table 1). In duloxetine-pretreated rats, oxaliplatin did not affect either PM or AM corticosterone levels, at either the acute (PM: 48.71 ± 8.66 pg/ml [baseline] to 45.58 ± 4.16 pg/ml; AM: 31.70 ± 6.68 pg/ml [baseline] to 26.68 ± 9.80) or late time point (PM: 51.4 ± 10.32 pg/ml; AM: 28.08 ± 9.77 pg/ml), providing independent support for the suggestion that duloxetine anti-hyperalgesia is dependent on attenuation of neuroendocrine stress axis function.

Discussion

Chemotherapy, a mainstay for the treatment of most forms of cancer, has been given to millions of patients worldwide (Miller et al., 2019). However, as more effective chemotherapy increases long-term survival, a growing population of cancer patients are living with pain from chronic CIPN, which has a major negative impact on their quality of life (Staff et al., 2017). Duloxetine, a serotonin-norepinephrine reuptake inhibitor (SNRI), the only treatment for painful CIPN recommended by the American Society of Clinical Oncology (Smith et al., 2013, 2017; Hershman et al., 2014; Ewertz et al., 2015; Obata, 2017), is also used to treat pain syndromes induced or exacerbated by stress (Finnerup et al., 2015; Kisler et al., 2019; Rodrigues-Amorim et al., 2020). Since stress may contribute to the incidence, severity, and duration of CIPN (Kober et al., 2018; Miaskowski et al., 2018b), we evaluated the role of neuroendocrine stress axes in the response of CIPN to duloxetine. Our findings not only demonstrate a contribution of a novel mechanism of duloxetine analgesia (anti-hyperalgesia), but also reveal a gonadal hormone dependence of this effect, which differs between oxaliplatin and paclitaxel CIPN.

It has been hypothesized that the analgesic effect of duloxetine is, like for other SNRIs, because of inhibition of presynaptic reuptake of two key pain inhibiting neurotransmitters, serotonin and noradrenaline, in the CNS, producing analgesia by enhancing descending pain inhibitory controls (Obata, 2017). However, unlike opioid analgesics, which act by enhancing descending antinociceptive controls (Millan, 2002), increasing nociceptive thresholds (Millan, 2002), duloxetine did not alone increase nociceptive threshold. Of note, in a randomized study of patients with depression, who were also experiencing pain, there was significant improvement in all pain measures when patients were switched from selective serotonin reuptake inhibitors to duloxetine (Perahia et al., 2009), supporting the importance of noradrenergic signaling to duloxetine. And while our findings do not exclude CNS-mediated effects of duloxetine on CIPN, our data support the hypothesis that duloxetine induces anti-hyperalgesia by modulating neuroendocrine stress axis function.

In a previous study, we demonstrated a contribution of neuroendocrine stress axes to CIPN (Ferrari et al., 2020). Now, in the current study, we demonstrate that duloxetine anti-hyperalgesia in CIPN is neuroendocrine stress axis-dependent. We

Table 1. Effect of duloxetine on change in plasma corticosterone induced by oxaliplatin[a]

	Oxaliplatin AM (ng/ml)	Oxaliplatin + duloxetine AM (ng/ml)	±Duloxetine difference	Oxaliplatin PM (ng/ml)	Oxaliplatin + duloxetine PM (ng/ml)	±Duloxetine difference
Acute	30.82 ± 6.371	−5.02 ± 12.76	$p = 0.025$*	8.75 ± 11.33	−3.13 ± 7.47	NS
Late	−4.99 ± 6.57	−5.14 ± 5.49	NS	22.38 ± 9.47	2.69 ± 5.25	NS

[a]To test the hypothesis that oxaliplatin-induced CIPN is dependent on activation of the HPA axis, we measured PM and AM plasma corticosterone concentrations before (baseline), 1 d (acute response) and 7 d (late response) after administration of oxaliplatin. There was an acute response increase (23.22 ± 9.59 ng/ml to 54.05 ± 10.1 ng/ml) of AM plasma corticosterone ($p = 0.0024$, one-tailed Student's t test), and an increase in late response (55.13 ± 9.22 ng/ml to 77.51 ± 12.99 ng/ml) of PM plasma corticosterone ($p = 0.0323$, one-tailed Student's t test); $n = 6$. Duloxetine inhibited oxaliplatin-induced acute AM increase in plasma oxaliplatin (31.70 ± 6.68 ng/ml to 26.68 ± 9.80 ng/ml), a significant inhibition (*$p = 0.025$). However, while the increase in plasma corticosterone in the late PM was attenuated by duloxetine (48.71 ± 8.66 ng/ml to 51.40 ± 10.32 ng/ml), the inhibition of the increase did not reach significance ($p = 0.067$, one-tailed Student's t test).

conducted experiments to test the hypothesis that the neuroendocrine stress axes contribute to CIPN, and observed that, when stress hormones, epinephrine and corticosterone, are clamped at a fixed level, duloxetine no longer attenuates oxaliplatin or paclitaxel CIPN (Fig. 5), suggesting that duloxetine's ability to attenuate chronic CIPN is dependent on its ability to attenuate neuroendocrine stress axis response induced by chemotherapy. In addition, we show that, when stress axes are tonically activated following unpredictable sound stress, duloxetine prevents the enhancement of CIPN by sound stress (duloxetine is equally effective in attenuating CIPN in sham sound stress vs sound stress groups; Fig. 6), again supporting the suggestion that duloxetine is attenuating stress axis response to chemotherapy. In further support of this suggestion, we observed that oxaliplatin-induces acute increase in AM corticosterone and late increase in PM corticosterone abolished in duloxetine-pretreated rats (Table 1). Of note, the daily peak in glucocorticoid levels occurs around the time of the habitual sleep-wake transition, the glucocorticoid awakening response, which in rats (nocturnal animals), is in the PM, and in humans in the AM. While clinically persistent stress can be associated with elevation of AM cortisol levels (Ockenfels et al., 1995; Starr et al., 2019), in other studies of persistent stress, investigators observed flattening of the cortisol slope (slower decay from AM peak to PM trough) (Adam et al., 2017; Mickle et al., 2020; Jones and Gwenin, 2021). Thus, the type of persistent stress may determine the nature of the change in HPA axis function. Of note, change in AM:PM glucocorticoid ratios is correlated with changes in mRNA expression in many glucocorticoid-responsive genes, including SGK1, UCP2, TNFα, and ANXA1 (Pavlatou et al., 2013) that are involved in pain pathways (Peng et al., 2012; Chen et al., 2014; Makker et al., 2017; Wu and Chen, 2019).

It has been hypothesized that pain associated with paclitaxel- and oxaliplatin-induced CIPN is related to mitochondrial damage secondary to oxidative stress, in DRG neurons (Zheng et al., 2011). Both paclitaxel and oxaliplatin induce apoptosis and cytotoxicity in DRG neurons by affecting the balance between pro- and anti-apoptotic BclII family proteins, with paclitaxel (Lu et al., 2020) and oxaliplatin (Scuteri et al., 2009) upregulating the pro-apoptotic protein Bax and downregulating the anti-apoptotic protein BclII, in DRG neurons. Glucocorticoid receptors form a complex with BclII, and high-dose corticosterone decreases both glucocorticoid receptors and BclII in cortical neurons (Du et al., 2009). Of note, duloxetine significantly inhibits the upregulation of Bax and downregulation of BclII produced by paclitaxel (Lu et al., 2020), providing a potential mechanism underlying duloxetine anti-hyperalgesia in CIPN; administration of duloxetine restored the Bax/BclII balance to attenuate this effect of stress.

Duloxetine attenuates pathologic changes in the peripheral nerve induced by paclitaxel (Lu et al., 2020) and oxaliplatin (Meng et al., 2019), and it would be of interest, in future studies, to examine the effect of duloxetine on the neurotoxic effects of chemotherapy to decreased epidermal nerve fiber density. However, such changes in epidermal nerve fiber density have been observed in rats beginning 15 d following oxaliplatin administration (Boyette-Davis and Dougherty, 2011) and beginning 14 d after paclitaxel administration (Boyette-Davis et al., 2011). However, as we observed hyperalgesia beginning 30 min after administration for oxaliplatin, and <2 d after paclitaxel, it is unlikely that mechanisms, such as decrease in fiber density, contribute to chemotherapy-induced hyperalgesia or the response to duloxetine.

Since pain activates neuroendocrine stress axes (Ulrich-Lai et al., 2006) and patients with CIPN have higher levels of anxiety, it would not be unexpected that duloxetine, in addition to impacting pain, could affect other manifestations of chemotherapy-induced peripheral neuropathy. Indeed, duloxetine has also been reported to attenuate chemotherapy-induced numbness (Smith et al., 2013, 2017; Knoerl, 2021) and tinnitus (Smith et al., 2013, 2017; Velasco et al., 2021), also common symptoms associated with oxaliplatin and paclitaxel chemotherapy, both of which can also be precipitated/exacerbated by stress (Amro et al., 2014; Miaskowski et al., 2018a, b; Hsu et al., 2020). Future studies will address this potential impact of duloxetine on other manifestations of chemotherapy-induced neurotoxicity.

We observed a significant sex difference in magnitude of the effect of duloxetine, which differed between oxaliplatin- and paclitaxel-induced CIPN. Duloxetine markedly attenuated oxaliplatin-induced hyperalgesia in males but produced a more modest attenuation in females, even when a higher dose of duloxetine was administered. In contrast, for paclitaxel CIPN, duloxetine more robustly reduced the hyperalgesia in females; and unlike for oxaliplatin CIPN, this sex difference was abrogated by increasing the dose of duloxetine in males. Why one can overcome the sex difference by increasing duloxetine dose in males with paclitaxel CIPN, but not in females with oxaliplatin, remains to be elucidated. While, to our knowledge, sex differences in response to duloxetine have not been studied in patients with CIPN, our data support the suggestion that this should be considered for future studies, to optimize the therapeutic use of duloxetine in treating CIPN pain. In the present experiments, we also found that the mechanisms underlying the sex differences in duloxetine anti-hyperalgesia, in rats with CIPN, are regulated by gonadal hormones, since prepubertal gonadectomy produced a switch in phenotype to one similar to that observed in the gonad-intact opposite sex. This may be especially relevant in the context of duloxetine's impact on neuroendocrine stress axes, since gonadal hormones differentially affect neuroendocrine stress axis function (Oyola and Handa, 2017). Supporting this suggestion, in previous studies, we demonstrated both that sympathoadrenal stress axis contributes to oxaliplatin CIPN, only in males (Staurengo-Ferrari et al., 2021), while in paclitaxel, a greater role of both stress axes is observed in females (Ferrari et al., 2020). Finally, as has been reported for analgesia induced by duloxetine and other SNRIs, in patients (Amr and Yousef, 2010; Li et al., 2021), the duration of duloxetine-

induced anti-hyperalgesia far exceeds its plasma half-life (6-7 h in Sprague Dawley rats) (Chae et al., 2013; Chen et al., 2019) in oxaliplatin- and paclitaxel-induced CIPN in rodents (Figs. 2 and 3). This phenomenon should be evaluated in the context of the effect of duloxetine on neuroendocrine stress axis function.

In conclusion, our work supports the suggestion that duloxetine-induced anti-hyperalgesia in CIPN is neuroendocrine stress axis-dependent, and that stress-induced enhancement of CIPN may be particularly responsive to treatment with duloxetine. Sex differences in duloxetine efficacy observed in our preclinical models of CIPN support the suggestion that sex as an experimental variable should be evaluated for in clinical studies, to help provide optimal treatment of the pain, and potentially other symptoms, associated with chemotherapy-induced peripheral neuropathy.

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