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Delta opioid receptors are essential to the antiallodynic action of B_2-mimetics in a model of neuropathic pain

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Abstract

The adrenergic system, because of its reported implication in pain mechanisms, may be a potential target for chronic pain treatment. We previously demonstrated that β_2-adrenoceptors (β_2-ARs) are essential for neuropathic pain treatment by antidepressant drugs, and we showed that agonists of β_2-ARs, that is, β_2-mimetics, had an antiallodynic effect per se following chronic administration. To further explore the downstream mechanism of this action, we studied here the role of the opioid system. We used behavioral, genetic, and pharmacological approaches to test whether opioid receptors were necessary for the antiallodynic action of a short acting (terbutaline) and a long-acting (formoterol) β_2-mimetic. Using the Cuff model of neuropathic pain in mice, we showed that chronic treatments with terbutaline (intraperitoneal) or formoterol (orally) alleviated mechanical hypersensitivity. We observed that these β_2-mimetics remained fully effective in μ-opioid and in κ-opioid receptor deficient mice, but lost their antiallodynic action in δ-opioid receptor deficient mice, either female or male. Accordingly, we showed that the δ-opioid receptor antagonist naltrindole induced an acute relapse of allodynia in mice with neuropathic pain chronically treated with the β_2-mimetics. Such relapse was also observed following administration of the peripheral opioid receptor antagonist naloxone methiodide. These data demonstrate that the antiallodynic effect of long-term β_2-mimetics in a context of neuropathic pain requires the endogenous opioid system, and more specifically peripheral δ-opioid receptors.

Keywords

β_2-mimetics, terbutaline, formoterol, neuropathic pain, mechanical allodynia, opioid system, δ-opioid

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Background

Antidepressant drugs including tricyclic antidepressants (TCAs) and selective serotonin-noradrenaline reuptake inhibitors (SSNRIs) are among the first-line treatments of neuropathic pain.[1–4] These drugs mainly act by blocking the transporters of noradrenaline and serotonin. The critical role of the noradrenergic component in neuropathic pain relief was first suggested based on the poor clinical effectiveness of selective serotonin reuptake inhibitors (SSRIs).[1–3,5] The lack of SSRIs action can also be observed preclinically in rodent models of neuropathic pain.[4,6] This raised the question of the potential role of the adrenoceptor(s), downstream of noradrenaline, in the action of the antidepressant drugs. We previously showed, by using a peripheral nerve injury model,[7] that the antiallodynic effect of

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chronic antidepressant drugs is mediated by β_2 adrenoceptors (β_2-ARs),[8–11] but not by α_2, nor β_1 or β_3-ARs. Following these findings, it has been suggested in animals that β_2-ARs agonists could be beneficial in peripheral neuropathic pain conditions.[12,13] These data were further supported by some clinical observations, showing in a case report that a β_2-mimetic induced significant relief in six patients with neuropathic pain,[14] and in a retrospective epidemiologic study that the relative incidence of post-thoracotomy neuropathic pain was five-fold lower for patients with a chronic β_2-mimetic treatment.[15]

Interestingly, the antidepressant drug mechanism leading to relief of mechanical hypersensitivity after chronic treatment is also delta-opioid (DOP) receptor dependent.[9,16–19]

The opioid system plays a leading role in inhibitory controls of pain.[20] It is involved in the direct action of analgesics targeting mu-opioid (MOP) receptors[21] such as morphine, but also in the indirect recruitment of opioid receptors in the action of antidepressant drugs against neuropathic pain.[16,22,23] In a similar way to antidepressant drugs, it has been suggested that the effect of β_2-mimetics on neuropathic mechanical allodynia may also involve the endogenous opioid system. Indeed, an acute administration of naltrindole, a DOP receptor antagonist, temporarily suppressed the benefit of chronic treatment with the very long-acting β_2-mimetic clenbuterol in a model of sciatic nerve compression,[12] and the effect of the β_2-mimetic terbutaline in a model of diabetic neuropathy.[24] These first data suggested that DOP receptors would be necessary for the therapeutic effect of β_2-mimetics on neuropathic pain, but still required to be genetically confirmed as well as to test the role of the other opioid receptors. Based on their half-life, β_2-mimetics can be classified as short-acting β_2-mimetics, such as terbutaline, salbutamol, fenoterol, and pirbuterol, which are prescribed for the curative treatment of asthma attacks and chronic obstructive pulmonary disease (COPD) or the threat of preterm labor; or as long-acting β_2-mimetics, such as formoterol and salmeterol, which are used as bronchodilators to help controlling and preventing COPD symptoms. Using transgenic and pharmacological approaches, we evaluated here the role of the three opioid receptors in the antiallodynic action of two β_2-mimetics, one with a short half-life, terbutaline (5–6 h), and the other with a long half-life, formoterol (12 h).[25]

In the present study, independently of their half-life, both chronic terbutaline and formoterol reversed mechanical hypersensitivity in our model of neuropathic pain. Using genetic and pharmacological approaches, we also showed that DOP receptors, but neither MOP nor kappa-opioid (KOP) receptors, are necessary for this action of β_2-mimetics.

Methods

Animals

Experiments were performed using C57BL/6J mice (Charles River, L'Arbresle, France) between 8 and 10 weeks old at the time of surgery and in mice deficient for MOP, DOP or KOP receptors, or β_2-ARs and their littermate controls. The generation of mice lacking MOP, DOP or KOP receptors, or β_2-ARs has been previously described.[26–29] Heterozygote mice were bred in our animal facilities and genotyping of the litters was done upon weaning. Experiments using transgenic mice were conducted on adult male and female wild type and knockout littermate mice (for formoterol experiments, to refine the use of animals and test gender aspects) or on male only (for terbutaline experiments), weighing 20–30 g. When we used male and female, each experimental group contained the same number of males and females. As the wild-type animals have the same background and the same behavior, they were pooled to form the control groups. For other experiments with C57BL/6J mice, we used males only. Mice were group-housed two to five per cage and kept under a 12-h light/dark cycle with food and water ad libitum. A total of 40 C57BL/6J mice, 44 MOP, 43 DOP, 44 KOP, and 24 β_2-AR-related mice were used for the experiments. Experiments were performed in agreement with European guidelines (EU 2010/63) and under protocols approved by the "Comité d'Ethique en Matière d'Expérimentation Animale de Strasbourg" (CREMEAS, CEEA35). At the end of the experiments, mice were killed by cervical dislocation according to institutional ethical guidelines. The animal facilities Chronobiotron UMS3415 are registered for animal experimentation under the Animal House Agreement A67-2018–38.

Model of neuropathic pain

Neuropathic pain was induced by cuffing the main branch of the right sciatic nerve.[7,30] Surgeries were performed under ketamine (68 mg/kg)/xylazine (10 mg/kg) intraperitoneal (i.p.) anesthesia (Centravet, Tadden, France). The common branch of the right sciatic nerve was exposed and a cuff of PE-20 polyethylene tubing (Harvard Apparatus, Les Ulis, France) of standardized length (2 mm) was unilaterally inserted around it (Cuff group). The shaved skin was closed using suture. Sham-operated mice underwent the same surgical procedure without implantation of the Cuff (Sham group). All treatments started between two and three weeks after the surgery.

Measure of mechanical allodynia

Mechanical allodynia was tested using von Frey hairs and results were expressed in grams. Tests were done

during the morning, starting at least 2 h after lights on. Mice were placed in clear Plexiglas boxes (7 cm × 9 cm × 7 cm) on an elevated mesh screen. Calibrated von Frey filaments (Bioseb, Vitrolles, France) were applied to the plantar surface of each hind-paw until they just bent, in a series of ascending forces up to the mechanical threshold. Filaments were tested five times per paw and the paw withdrawal threshold (PWT) was defined as the lower of two consecutive filaments for which three or more withdrawals out of the five trials were observed.[30,31] The person who conducted the tests was blinded to the treatments.

Treatment procedures

The terbutaline treatment began 15 days after the neuropathy was induced, and it was maintained at least three weeks. During the treatment, mice received two intraperitoneal injections per day (morning and evening) of terbutaline hemisulfate (0.5 mg/kg, 5 mL/kg, Sigma-Aldrich, St. Quentin Fallavier). The dose was chosen based on a previous dose–response experiment.[13] The drug was dissolved in 0.9% NaCl solution that was also used for control injections. The injection of naltrindole (5 mg/kg, subcutaneous, Sigma-Aldrich), a DOP receptors antagonist, was done 36 days after surgery, that is, after 21 days of terbutaline treatment, in wild-type, MOP and KOP receptors-deficient mice. The injection of naloxone methiodide (5 mg/kg, subcutaneous, Sigma-Aldrich), an opioid receptor antagonist that does not cross the blood–brain barrier, was done following the same procedure as for naltrindole, but in C57BL/6J mice. In order to evaluate the mechanical thresholds for nociceptive response, mice were submitted to von Frey tests before and 30 min after the antagonist injection.

Formoterol (Biotrend Chemicals AG, Zürich, Switzerland) was delivered per os through the drinking water with ad libitum access as sole source of fluid. The drug was dissolved in water with 0.02% saccharin to increase palatability and control mice received a solution of 0.02% saccharin in water (vehicle solution). To assess the daily dose of β_2-mimetic that was actually received by the animals (in μg/kg/day), the bottles containing the treatment were regularly weighed and we calculated the ratio between the intake amount of formoterol per cage and the weight of the animals. A first set of experiments (Figure 3) included mice treated with oral formoterol at concentrations 0.5, 0.1, 0.05, or 0.001 μg/mL and their controls, and Sham mice that received the highest dose of formoterol (0.5 μg/mL) to test whether it had an analgesic effect per se (n = 5 in each group).

Mice lacking MOP, DOP, or KOP receptors received formoterol treatment (0.5 μg/mL), in the drinking water, following the same protocol. Data were pooled from four independent experiments, due to the non-regular production of mouse breeding. Four additional sets of mice were also used to pharmacologically assess the role of opioid receptors. Each set was composed of three groups, a Sham and a Cuff group treated with the oral vehicle, and a Cuff group treated with formoterol (0.5 μg/mL). After three weeks of oral treatment, mice received a subcutaneous injection of the MOP receptor antagonist naloxonazine (Sigma-Aldrich, 30 mg/kg) or the DOR receptor antagonist naltrindole (5 mg/kg) or a saline solution (0.9% NaCl). All drugs were dissolved in NaCl 0.9%. In order to evaluate the mechanical threshold, mice were submitted to von Frey testing before, 30 min and 1 h after the antagonist injection.

Data and statistical analysis

Data are expressed as mean ± SEM. In Figures 1(c) and 3 (b), cumulated PWTs during treatments were quantified as the area under the curve above 0 (AUC), calculated by the trapezoidal method.[32] Statistical analyses were performed with STATISTICA 10 (Statsoft, Tulsa, OK, USA) using multifactor analysis of variance. The surgery procedure (Sham or Cuff) and the treatments were taken as between-group factors. When needed, the time of measurement (either time course or preinjection vs. postinjection data) was taken as a within-subject factor. The Duncan test was used for post hoc comparisons. The significance level was set at $p < 0.05$.

Results

Terbutaline treatment in opioid receptor deficient mice

Cuff-implantation induced an ipsilateral mechanical hypersensitivity in wild-type mice, as previously described[7,16–18,30] (Figure 1(a); $F_{7,231} = 10.25$, $p < 0.001$; detailed statistics for all results are given in Table 1 in Supplemental Material). As previously showed,[9,16–19] this induction and maintenance of mechanical hypersensitivity was also similar along the experiment between wild-type, $MOP^{-/-}$, $DOP^{-/-}$ and $KOP^{-/-}$ mice (Figure 1(b)) (even though enhanced allodynia between $DOP^{-/-}$ and their wild-type controls has been reported by others in the partial ligation model).[33] Two weeks after cuff insertion, we started the treatments with terbutaline (0.5 mg/kg) or the control saline solution (0.9% NaCl), twice a day. Terbutaline treatment alleviated the cuff-induced allodynia in wild-type mice after about 13 days of treatment (Figure 1(a)). The same anti-allodynic effect was also present in MOP (Figure 1(c); $F_{3,23} = 23.82$, $p < 0.001$) and KOP receptor-deficient mice (Figure 1(c); $F_{3,23} = 23.82$, $p < 0.001$), while the terbutaline treatment was ineffective in DOP receptor-deficient mice (Figure 1(c); $F_{3,23} = 23.82$, $p < 0.001$).

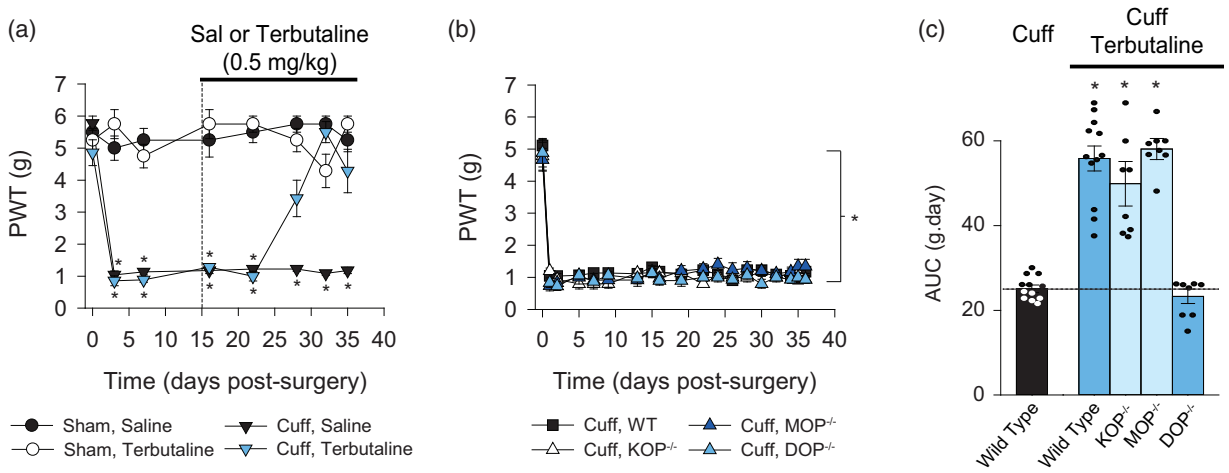

Figure 1. DOP receptors, but not MOP or KOP receptors, are critical to terbutaline treatment of mechanical allodynia. Two weeks after unilateral surgery on the right hindpaw, terbutaline treatment (0.5 mg/kg, i.p., twice a day) or its saline control solution started and continued until day 36 post-surgery. The mechanical threshold of hindpaw withdrawal was evaluated using von Frey filaments. (a) Chronic terbutaline treatment reversed the mechanical hypersensitivity in wild-type mice (n = 8–9 per group, $*p < 0.01$ compared to Sham-operated controls receiving saline). (b) Wild-type, $MOP^{-/-}$, $DOP^{-/-}$ and $KOP^{-/-}$ mice displayed the same baseline for mechanical sensitivity and the same cuff-induced mechanical hypersensitivity (n = 6–9 per group, $*p < 0.001$ for post-operative day 0 (baseline) compared to others post-operative days in each group). (c) AUC from post-surgical day 15 (first day of treatment) until the end of treatment, for wild-type, $MOP^{-/-}$, $DOP^{-/-}$, and $KOP^{-/-}$ mice. The chronic terbutaline treatment suppressed the ipsilateral cuff-induced allodynia in MOP and KOP receptor-deficient mice but remained ineffective in DOP receptor-deficient mice (n = 8–12 per group, $*p < 0.01$ compared to WT mice receiving Saline). Data are expressed as mean ± SEM, individual values are also displayed for the AUC. PWT: paw withdrawal threshold; AUC: area under the curve; KOP: kappa-opioid; MOP: mu-opioid; DOP: delta-opioid.

To confirm this critical role of DOP receptors, we then tested the consequences of an acute injection of naltrindole in the wild-type, MOP and KOP receptor-deficient mice of the above experiment. After three weeks of treatment with terbutaline or saline, an acute injection of naltrindole induced a relapse of mechanical hypersensitivity in these mice (Figure 2(a); WT: $F_{1,33} = 18.83$, $p < 0.001$; $MOP^{-/-}$: $F_{1,21} = 21.61$, $p < 0.001$; $KOP^{-/-}$: $F_{1,20} = 5.05$, $p < 0.01$). We also controlled that naltrindole per se had no effect in mice with Sham surgery or in neuropathic mice treated with saline.

Peripherals opioid receptors are involved in terbutaline antiallodynic effect

The acute injection of naloxone methiodide (5 mg/kg), an opioid receptor antagonist that does not cross the blood–brain barrier, induced an acute relapse of allodynia in cuff-implanted mice chronically treated with terbutaline (Figure 2(b); $F_{1,20} = 10.9$, $p < 0.01$). The same dose of naloxone methiodide had no effect in mice with Sham surgery treated with saline.

Antiallodynic action of chronic oral formoterol: dose response

For treatment with the long-acting β_2-mimetic formoterol, we chose to use chronic oral administration, which first required determining the appropriate dose (Figure 3). Cuff implantation induced an ipsilateral mechanical allodynia (Figure 3(a); $F_{11,242} = 5.8$, $p < 0.001$). We did not observe any change in the nociceptive threshold on the left paw or in the Sham group. Nineteen days after surgery, we started treatments with formoterol (0.001, 0.05, 0.1, or 0.5 µg/mL) or with saccharin vehicle solution (0.02% saccharin). Chronic formoterol treatment at doses 0.05, 0.1, and 0.5 µg/mL alleviated the cuff-induced allodynia after 19 days, 15 days, and 13 days of treatment respectively (Figure 3 (a); $F_{11,242} = 5.8$, $p < 0.001$). The 0.001 µg/mL dose of formoterol had no significant effect; and treatments at different doses did not affect the contralateral nociceptive thresholds (left paw, Figure 3(a)). The AUC for the treatment period is presented in Figure 3(b). The drinking bottles were regularly weighed during the experiment. Considering the volume of solution drank by the mice per 24 h (between 3.5 and 5 mL per mouse), the 0.001 µg/mL solution was equivalent to 2.0 ± 0.1 µg/kg/day, the 0.05 µg/mL solution was equivalent to 9.8 ± 0.5 µg/kg/day, the 0.1 µg/mL solution was equivalent to 17.8 ± 1.0 µg/kg/day, and the 0.5 µg/mL solution was equivalent to 72.7 ± 4.8 µg/kg/day (Figure 3(c)). These amounts were in fact mostly taken over the 12-h night period, period during which mice usually drink.

To test whether formoterol had an analgesic effect per se, we tested the higher dose (0.5 µg/mL) in Sham

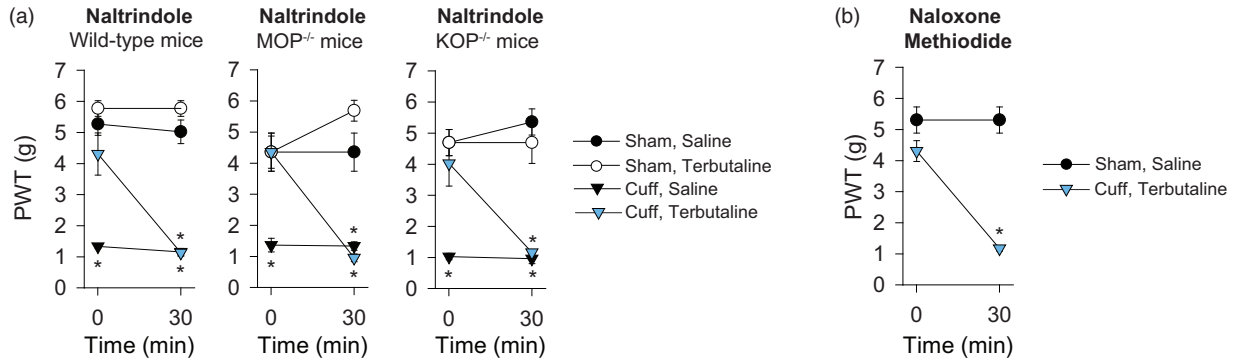

Figure 2. Pharmacological blockade of DOP receptors induced a relapse of allodynia in terbutaline-treated mice. After three weeks of chronic terbutaline treatment (0.5 mg/kg, i.p., twice a day), mice received an acute injection of opioid receptor antagonists. (a) The acute administration of naltrindole (5 mg/kg, s.c.), a DOP receptor antagonist, suppressed the antiallodynic effect of terbutaline in wild-type, $MOP^{-/-}$ and $KOP^{-/-}$ mice. (b) The acute administration of naloxone methiodide (5 mg/kg, s.c.), an opioid receptor antagonist that does not cross the blood-brain barrier, induced a relapse of allodynia in terbutaline treated mice. Mice were tested before (0) and 30 min after the acute injection ($n = 8$–12 per group, $^*p < 0.05$ compared to Sham-operated controls chronically treated with saline). Data are expressed as mean ± SEM. PWT: paw withdrawal threshold; KOP: kappa-opioid; MOP: mu-opioid.

control mice. This chronic oral treatment did not affect mechanical thresholds of the Sham group (Figure 3(d)).

Chronic oral formoterol treatment in opioid and β_2-adrenergic receptor deficient mice

As previously observed,[9,34] mechanical sensitivity thresholds of female mice were significantly lower than in males (baseline thresholds: 3.4 g ± 0.7 for females, 4.9 g ± 0.9 for males, $t_{11} = 4.18$, $p < 0.005$). Both male and female mice developed mechanical allodynia after surgery, and formoterol treatment suppressed the cuff-induced allodynia in both sexes (Figure 4(a); female mice: $F_{10,90} = 2.2$, $p < 0.01$; male mice: $F_{10,90} = 5.4$, $p < 0.001$). Groups including the same number of male and female mice were pooled for data presentation (Figure 4(b) to (d)).

MOP-, DOP-, and KOP-receptors or β_2-AR deficient mice displayed the same baseline for mechanical sensitivity and cuff-induced mechanical hypersensitivity as their wild-type littermates, as previously observed.[9–11,16–18] Formoterol treatment alleviated cuff-induced allodynia in wild-type mice (Figure 4(b); $F_{10,210} = 5.6$, $p < 0.001$). The same antiallodynic effect was also present in MOP (Figure 4(c); $F_{10,150} = 5.5$, $p < 0.001$) and KOP receptor-deficient mice (Figure 4(c); $F_{10,150} = 4.1$, $p < 0.001$), while formoterol treatment was ineffective in DOP receptor-deficient mice (Figure 4(c); $F_{10,150} = 2.7$, $p < 0.001$), and in β_2-AR deficient mice (Figure 4(d); $F_{10,150} = 1.8$, $p < 0.05$).

Effect of opioid receptor antagonists on long lasting formoterol treatment

To confirm the role of DOP receptors, independently from any developmental or lasting alteration of nociceptive pathway that may accompany opioid receptor deletion, we tested the consequence of an acute injection of the MOP receptor antagonist naloxonazine and the DOP receptor antagonist naltrindole (Figure 5). After three weeks of treatment with formoterol or saccharin, an acute injection of naltrindole temporarily suppressed the antiallodynic effect of formoterol treatment (Figure 5; $F_{2,24} = 5.3$, $p < 0.005$), while naloxonazine had no effect on mechanical thresholds for paw withdrawal. We also observed that naltrindole had no effect per se in mice with Sham surgery or in mice that received saccharin alone.

Discussion

In the present work, we assessed the role of the opioid receptors in the antiallodynic effect of two β_2-mimetics, one with a short half-life, terbutaline, given intraperitoneally, and the other one with a long half-life, formoterol, given orally. We found that DOP receptors, but not MOP or KOP receptors, were required to the effect of those two β_2-mimetics on mechanical hypersensitivity in a murine model of neuropathic pain.

Regardless of their half-life, terbutaline and formoterol exerted their effect with a similar therapeutic delay of about 13 days, which is also similar to the therapeutic delay of treatments with antidepressant drugs in the same model.[9–11] Present data with oral formoterol and previous reports with sustained systemic injections of β-mimetics[12,13] also suggest that the dose, once effective, has only a minor influence on this delay. The delay of action of these treatments in the context of neuropathic pain thus appears partly independent from the pharmacokinetic properties of the molecules and ways of administration, supporting the hypothesis that the

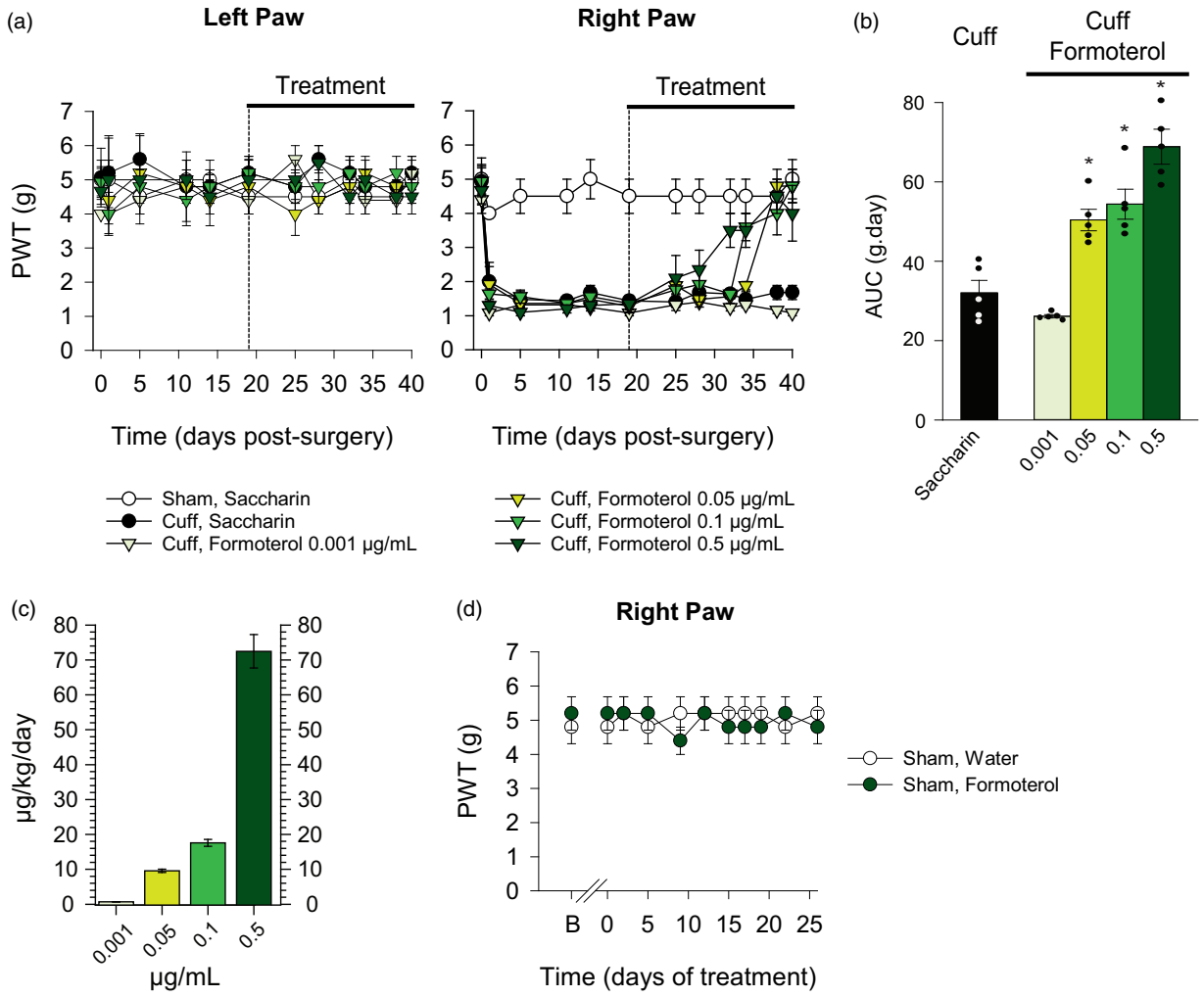

Figure 3. Chronic oral formoterol treatment suppresses cuff-induced mechanical allodynia. Nineteen days after unilateral surgery on the right hindpaw, the chronic oral treatment with formoterol started and lasted 21 days. (a) The animals (n = 5 per group) received formoterol (0.001, 0.05, 0.1, or 0.5 µg/mL) with saccharin 0.02% or saccharin alone in the drinking water as the sole source of fluid. The mechanical sensitivity (PWTs) was evaluated over days using von Frey filaments. Control treatment (saccharin 0.02%) did not affect the mechanical sensitivity. Chronic oral formoterol treatment at the three highest doses reversed the cuff-induced allodynia. The treatment at dose 0.001 µg/mL had no effect. (b) AUC from for post-surgical day 19 (first day of treatment) until the end of the treatment for formoterol (0.001, 0.05, 0.1, or 0.5 µg/mL). *$p < 0.05$ compared to cuff-operated control group drinking vehicle. (c) Histogram showing the equivalence between µg/mL and µg/kg/day of the different doses. (d) Formoterol treatment (0.5 µg/mL) did not affect mechanical sensitivity of Sham-operated mice. Data are expressed as mean ± SEM, individual values are also displayed for AUC. PWT: paw withdrawal threshold.

delay may be more likely linked to the setting up of molecular and cellular neuroplasticity phenomena, as it has been described with antidepressant drugs in the context of depression.[35–37]

β_2-mimetics remained effective in mice deficient for MOP or KOP receptors, but lost their efficacy in mice deficient for DOP receptors, which seemed the sole opioid receptors necessary to the antiallodynic effect of β_2-mimetics. This finding is further supported by pharmacological data. Indeed, the acute injection of the DOP receptor antagonist naltrindole led to a transitory relapse of mechanical allodynia in mice chronically treated with β_2-mimetics. These results suggest a mechanism similar to the one previously observed for antidepressant drugs, whose antiallodynic action has also been shown to be dependent upon DOP receptors.[9,16,19] In the Cuff model, it was also shown that sustained neuropathic pain condition was associated with a reduction of DOP receptor expression in small calcitonin gene-related peptide positive primary sensory neurons and in free nerve endings, that was not present in duloxetine[19] treated animals and that was partly corrected in

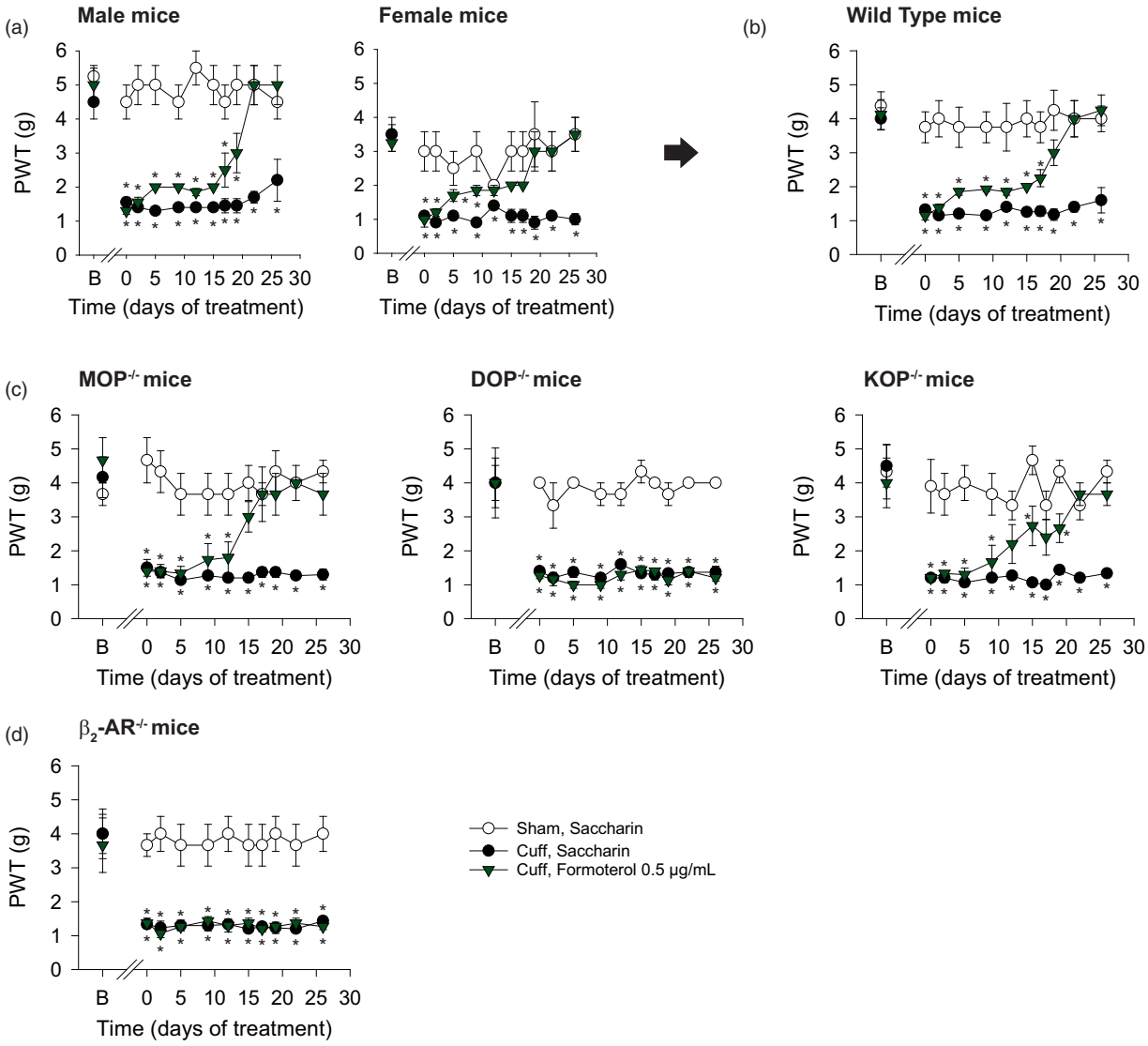

Figure 4. DOP receptors, but not MOP or KOP receptors, are critical to formoterol treatment of mechanical allodynia. Between 15 and 19 days post-surgery, the oral treatment with formoterol (0.5 µg/ml) or its saccharin 0.02% solution control started and was maintained for over three weeks. Mechanical allodynia was tested using von Frey filaments. (a) The mechanical sensitivity thresholds (PWT) of female mice is lower than that of male mice. However, both sexes developed mechanical allodynia similarly, and formoterol was effective in reversing the cuff-induced allodynia in both male and female mice. Males and females were then pooled in each experimental group, with an equal number of mice of both sexes in each group. (b and c) Chronic formoterol treatment suppressed the ipsilateral cuff-induced allodynia in wild-type mice, as well as in MOP and KOP receptor-deficient mice, but it remained ineffective in DOP receptor-deficient mice. (d) Formoterol had no action on mechanical allodynia in β_2-AR deficient mice. (n = 3–4 males and 3–4 females per group, $^*p < 0.05$ compared to Sham-operated control group drinking vehicle). Data are expressed as mean ± SEM. PWT: paw withdrawal threshold; AUC: area under the curve; KOP: kappa-opioid; MOP: mu-opioid; DOP: delta-opioid.

formoterol[38] treated animals. In physiological conditions, DOP receptor agonists have little noticeable effect on nociception, as shown by pharmacological studies and data in DOP receptor deficient mice.[39] However, under neuropathic pain conditions, the administration of DOP receptor agonists has been shown to decrease mechanical and thermal pain modalities.[40–44] It has thus been suggested that DOP agonists may be of interest for pain treatment. Unfortunately, agonists of the DOP receptors cannot be presently used as appropriate clinical treatments for neuropathic pain. Indeed, while DOP agonists would have a lower abuse potential and life-threatening risk than MOP receptor agonists,[39,45] the molecules presently available have adverse effects,[46] in particular convulsions or sleep apnea, that prevent their clinical use.

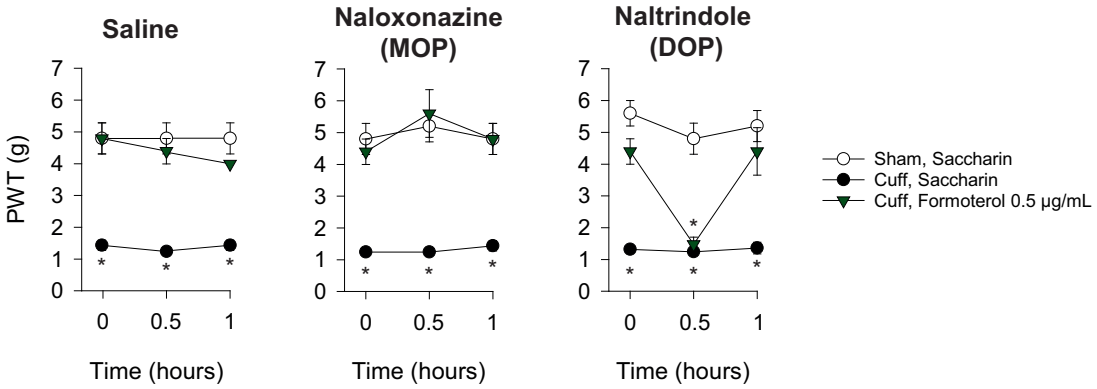

Figure 5. Pharmacological blockade of DOP receptors induced a relapse of allodynia in formoterol-treated mice. After at least three weeks of formoterol or saccharin treatment, mice received an injection of the MOP receptor antagonist naloxonazine (30 mg/kg, s.c.), DOP receptor antagonist naltrindole (5 mg/kg, s.c.), or their control saline solution. The mechanical threshold was measured before, 30 min and 1 h after acute injection. Naltrindole, but not naloxonazine, induced a transitory relapse of mechanical allodynia in neuropathic mice treated by formoterol. Data are expressed as mean ± SEM ($n = 5$ per group, $^*p < 0.001$ compared to Sham-operated control group drinking vehicle). PWT: paw withdrawal threshold; MOP: mu-opioid; DOP: delta-opioid.

β_2-AR and DOP receptors are both necessary to the antiallodynic effect of antidepressant drugs[10,11,16] and of β_2-mimetics. Interestingly, the contribution of these two receptors to an antinociceptive action has also been reported following intraplantar administration of a β-AR agonist in inflammatory pain[47] as well as in the antiallodynic action of a prolonged treatment with curcuma in a model of sciatic nerve constriction.[48] The mechanistic link between these two receptors is however still to be explored. Since acute administration of naloxone methiodide, an antagonist which does not pass the blood-brain barrier, suppressed the antiallodynic effect of chronic terbutaline treatment, the peripheral opioid system can thus be considered as critical. Again, this peripheral DOP receptor component has also been observed concerning the antiallodynic action of prolonged treatment with antidepressant drugs.[9,19] DOP receptors are present on primary afferents[47,49,50] and expressed by several classes of dorsal root ganglia neurons.[19,51] On the other hand, the β_2-ARs, whose mRNA levels may increase in the dorsal root ganglia following sciatic nerve transection[52] but not after sciatic nerve cuffing,[8] were proposed to be preferentially expressed by the glial satellite cells of dorsal root ganglia.[8] These non-neuronal cells are the peripheral analogues of astrocytes of the central nervous system and are known to express β_2-AR at their membrane in various species including humans.[53,54] Even though, in transfected cell cultures, the existence of heteromeric complexes of DOP receptors and β_2-AR has been studied,[55–57] the above anatomical evidence suggests that both receptors would unlikely be co-expressed by the same cells in dorsal root ganglia. The immediate relapse after an acute administration of naltrindole rather suggests that the opioid component is likely located downstream of the adrenergic component. We may thus hypothesize that the recruitment of β_2-AR could lead to an activation of the cAMP/PKA pathway in satellite glial cells, which could lead to synthesis and release of opioid peptides like enkephalins that would then stimulate DOP receptors present on primary afferents.[47,50] Alternately, it could also be hypothesized that DOP receptors are simply part of a permissive mechanism, in which their presence would be essential to the normal functioning of pathways responsible for the antiallodynic effect, without being per se an element of the molecular therapeutic cascade itself. This hypothesis could be supported by the fact that DOP receptors were shown to be more particularly involved in mechanical nociceptive responses.[58]

Conclusion

In summary, the present findings show that β_2-mimetics, either intraperitoneal or per os, can have an antiallodynic action in a rodent model of sciatic nerve compression, and that DOP receptors are essential to it. Our results also show that neither MOP nor KOP receptors are necessary to this action of β_2-mimetics. Our data are also supportive of a peripheral location of the opioid component of β_2-mimetic effect. Together, these findings show a mechanistic convergence between β_2-mimetics and the known antidepressant drugs' action in neuropathic pain models. These findings also support the need of future research to elucidate the mechanistic link between β_2-ARs and DOP receptors.

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Author Contributions

MK, SM, and IY did all surgeries. SM and YB did all experiments concerning terbulatine effect. For formoterol experiments, MK, XW, LN, and RAC performed dose-responses. MK, LN, and XW performed behavioral tests on chronically treated opioid-receptor deficient mice. MK performed behavioral tests concerning naloxonazine and naltrindole. SD genotyped the transgenic animals. MB, MK, DM, IY, and ES codesigned and supervised all experiments. MK collected and analyzed all data. MK and MB drafted the article.

Declaration of Conflicting Interests

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Supplemental Material

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