Delta opioid receptors are essential to the antiallodynic action of B2-mimetics in a model of neuropathic pain

Mélanie Kremer¹, Salim Megat¹, Yohann Bohren¹, Xavier Wurtz¹, Laurent Nexon¹, Rhian Alice Ceredig¹, Stéphane Doridot², Dominique Massotte¹, Eric Salvat¹,³, Ipek Yalcin¹, and Michel Barrot¹

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 β2-adrenoceptors (β2-ARs) are essential for neuropathic pain treatment by antidepressant drugs, and we showed that agonists of β2-ARs, that is, β2-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) β2-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 β2-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 β2-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 β2-mimetics in a context of neuropathic pain requires the endogenous opioid system, and more specifically peripheral δ-opioid receptors.

Keywords
β2-mimetics, terbutaline, formoterol, neuropathic pain, mechanical allodynia, opioid system, δ-opioid

Date Received: 20 November 2019; revised: 17 January 2020; accepted: 30 January 2020

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...
chronic antidepressant drugs is mediated by \( \beta_2 \) adrenoceptors (\( \beta_2 \)-ARs)\(^8\)\(^–\)\(^11\) but not by \( \alpha_2 \), nor \( \beta_1 \) or \( \beta_3 \)-ARs. Following these findings, it has been suggested in animals that \( \beta_2 \)-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 \( \beta_2 \)-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 fivefold lower for patients with a chronic \( \beta_2 \)-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 \( \beta_2 \)-mimetics on neuropathic mechanical allodynia may also involve the endogenous opioid system. Indeed, an acute administration of naltrexone, a DOP receptor antagonist, temporarily suppressed the benefit of chronic treatment with the very long-acting \( \beta_2 \)-mimetic clenbuterol in a model of sciatic nerve compression,\(^12\) and the effect of the \( \beta_2 \)-mimetic terbutaline in a model of diabetic neuropathy.\(^24\) These first data suggested that DOP receptors would be necessary for the therapeutic effect of \( \beta_2 \)-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, \( \beta_2 \)-mimetics can be classified as short-acting \( \beta_2 \)-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 \( \beta_2 \)-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 \( \beta_2 \)-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 \( \beta_2 \)-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 \( \beta_2 \)-ARs and their littermate controls. The generation of mice lacking MOP, DOP or KOP receptors, or \( \beta_2 \)-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 \( \beta_2 \)-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 DOR receptor 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 testing 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 β2-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 described7,16–18,30 (Figure 1(a); F2,231 = 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 allostynia 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 allostynia 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); F3,23 = 23.82, p < 0.001) and KOP receptor-deficient mice (Figure 1(c); F3,23 = 23.82, p < 0.001), while the terbutaline treatment was ineffective in DOP receptor-deficient mice (Figure 1(c); F3,23 = 23.82, p < 0.001).
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 β2-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...
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 \(\beta_2\)-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 \(\pm\) 0.7 for females, 4.9 g \(\pm\) 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 \(\beta_2\)-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 \(\beta_2\)-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 \(\beta_2\)-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 \(\beta_2\)-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 \(\beta\)-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
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

β2-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 β2-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 β2-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 duloxetine19 treated animals and that was partly corrected in
formoterol 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. However, under neuropathic pain conditions, the administration of DOP receptor agonists has been shown to decrease mechanical and thermal pain modalities. 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, the molecules presently available have adverse effects, in particular convulsions or sleep apnea, that prevent their clinical use.

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 mg/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 β2-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.
β2-AR and DOP receptors are both necessary to the antiallodynic effect of antidepressant drugs\textsuperscript{10,11,16} and of β2-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\textsuperscript{47} as well as in the antiallodynic action of a prolonged treatment with curcuma in a model of sciatic nerve constriction.\textsuperscript{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.\textsuperscript{9,19} DOP receptors are present on primary afferents\textsuperscript{47,49,50} and expressed by several classes of dorsal root ganglia neurons.\textsuperscript{19,51} On the other hand, the β2-ARs, whose mRNA levels may increase in the dorsal root ganglia following sciatic nerve transection\textsuperscript{52} but not after sciatic nerve cuffing,\textsuperscript{8} were proposed to be preferentially expressed by the glial satellite cells of dorsal root ganglia.\textsuperscript{8} These non-neuronal cells are the peripheral analogues of astrocytes of the central nervous system and are known to express β2-AR at their membrane in various species including humans.\textsuperscript{53,54} Even though, in transfected cell cultures, the existence of heteromeric complexes of DOP receptors and β2-AR has been studied\textsuperscript{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 β2-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.\textsuperscript{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.\textsuperscript{58}

**Conclusion**

In summary, the present findings show that β2-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 β2-mimetics. Our data are also supportive of a peripheral location of the opioid component of β2-mimetic effect. Together, these findings show a mechanistic convergence between β2-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 β2-ARs and DOP receptors.

**Acknowledgment**

The authors are grateful to Profs. Brigitte Kieffer and Claire Gavériaux-Ruff (Strasbourg, France) for providing MOP, DOP, and KOP-related mouse breeders.
Author Contributions
MK, SM, and IY did all surgeries. SM and YB did all experiments concerning terbutaline 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
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by the Centre National de la Recherche Scientifique (contracts UPR3212 and UMR5293), the University of Strasbourg and the Agence Nationale de la Recherche (Euridol ANR-17-EURE-0022); MK was supported by a CNRS disability doctoral fellowship, the Fondation d’Entreprise Banque Populaire, and the Fédération Hewlett Packard.

ORCID iD
Mélanie Kremer https://orcid.org/0000-0003-2814-5706

Supplemental Material
Supplemental material for this article is available online.

References
1. Attal N, Cruccu G, Baron R, Haanpaa M, Hansson P, Jensen TS, Nurmikko T. European Federation of Neurological Societies. EFNS guidelines on the pharmacological treatment of neuropathic pain: 2010 revision. Eur J Neurol 2010; 17: e113–e88.
2. Dworkin RH, O’Connor AB, Audette J, Baron R, Gourlay GK, Haanpää ML, Kent JL, Krane EJ, Lebel AA, Levy RM, Mackey SC, Mayer J, Miskowski C, Raja SN, Rice ASC, Schmader KE, Stacey B, Stanos S, Treede RD, Turk DC, Walco GA, Wells CD. Recommendations for the pharmacological management of neuropathic pain: an overview and literature update. Mayo Clin Proc 2010; 85: S3–S14.
3. Saarto T, Wiffen PJ. Antidepressants for neuropathic pain: a Cochrane review. J Neurol Neurosurg Psychiatry 2010; 81: 1372–1373.
4. Kremer M, Salvat E, Muller A, Yalcin I, Barrot M. Antidepressants and gabapentinoids in neuropathic pain: mechanistic insights. Neuroscience 2016; 338: 183–206.
5. Max MB, Lynch SA, Muir J, Shoaf SE, Smoller B, Dubner R. Effects of desipramine, amitriptyline, and fluoxetine on pain in diabetic neuropathy. N Engl J Med 1992; 326: 1250–1256.
6. Benbouzid M, Choucair-Jaafar N, Yalcin I, Waltisperger E, Muller A, Freund-Mercier MJ, Barrot M. Chronic, but not acute, tricyclic antidepressant treatment alleviates neuropathic allodynia after sciatic nerve cuffing in mice. Eur J Pain 2008; 12: 1008–1017.
7. Benbouzid M, Pallage V, Rajalu M, Waltisperger E, Doridot S, Poisbeau P, Freund-Mercier MJ, Barrot M. Sciatic nerve cuffing in mice: a model of sustained neuropathic pain. Eur J Pain 2008; 12: 591–599.
8. Bohren Y, Tessier LH, Megat S, Petitjean H, Hugel S, Daniel D, Kremer M, Fournel S, Hein L, Schlüchter R, Freund-Mercier MJ, Yalcin I, Barrot M. Antidepressants suppress neuropathic pain by a peripheral beta2-adrenoceptor mediated anti-TNFalpha mechanism. Neurobiol Dis 2013; 60: 39–50.
9. Kremer M, Yalcin I, Goumon Y, Wurtz X, Nexon L, Daniel D, Megat S, Ceredig RA, Ernst C, Turecki G, Chavant V, Theroux JF, Lacaud A, Joganah LE, Lelievre V, Massotte D, Lutz PE, Gilsbach R, Salvat E, Barrot M. A dual noradrenergic mechanism for the relief of neuropathic allodynia by the antidepressant drugs duloxetine and amitriptyline. J Neurosci 2018; 38: 9934–9954.
10. Yalcin I, Tessier LH, Petit-Demouliere N, Doridot S, Hein L, Freund-Mercier MJ, Barrot M. Beta2-adrenoceptors are essential for desipramine, venlafaxine or reboxetine action in neuropathic pain. Neurobiol Dis 2009; 33: 386–394.
11. Yalcin I, Choucair-Jaafar N, Benbouzid M, Tessier LH, Muller A, Hein L, Freund-Mercier MJ, Barrot M. Beta2-adrenoceptors are critical for antidepressant treatment of neuropathic pain. Ann Neurol 2009; 65: 218–225.
12. Yalcin I, Tessier LH, Petit-Demouliere N, Waltisperger E, Hein L, Freund-Mercier MJ, Barrot M. Chronic treatment with agonists of beta(2)-adrenergic receptors in neuropathic pain. Exp Neurol 2010; 221: 115–121.
13. Choucair-Jaafar N, Yalcin I, Rodenau JL, Waltisperger E, Freund-Mercier MJ, Barrot M. Beta2-adrenoceptor agonists alleviate neuropathic allodynia in mice after chronic treatment. Br J Pharmacol 2009; 158: 1683–1694.
14. Cok OY, Eker HE, Yalcin I, Barrot M, Aribogan A. Is there a place for beta-mimetics in clinical management of neuropathic pain? Salbutamol therapy in six cases. Anesthesiology 2010; 112: 1276–1279.
15. Salvat E, Schweitzer B, Massard G, Meyer N, de Blay F, Muller M, Barrot M. Effects of beta2 agonists on post-thoracotomy pain incidence. Eur J Pain 2015; 19: 1428–1436.
16. Benbouzid M, Gaverniaux-Ruff C, Yalcin I, Waltisperger E, Tessier LH, Muller A, Kieffer BL, Freund-Mercier MJ, Barrot M. Delta-opioid receptors are critical for tricyclic antidepressant treatment of neuropathic allodynia. Biol Psychiatry 2008; 63: 633–636.
17. Bohren Y, Karavelic D, Tessier LH, Yalcin I, Gaverniaux-Ruff C, Kieffer BL, Freund-Mercier MJ, Barrot M. Mu-opioid receptors are not necessary for
nortriptyline treatment of neuropathic allodynia. Eur J Pain 2010; 14: 700–704.
18. Megat S, Bohren Y, Doridot S, Gaveriaux-Ruff C, Kieffer BL, Freund-Mercier MJ, Yalcin I, Barrot M. kappa-Opioid receptors are not necessary for the antidepressant treatment of neuropathic pain. Br J Pharmacol 2015; 172: 1034–1044.
19. Ceredig RA, Pierre F, Doridot S, Alduntzin U, Salvat E, Yalcin I, Gaveriaux-Ruff C, Barrot M, Massotte D. Peripheral delta opioid receptors mediate duloxetine antiallodynic effect in a mouse model of neuropathic pain. Eur J Neurosci 2018; 48: 2231–2246.
20. Mogil JS, Yu L, Basbaum AI. Pain genes? Natural variation and transgenic mutants. Annu Rev Neurosci 2000; 23: 777–811.
21. Gaveriaux-Ruff C, Kieffer BL. Opioid receptor genes inactivated in mice: the highlights. Neuropeptides 2002; 36: 62–71.
22. Marchand F, Alloui A, Chapuy E, Jourdan D, Pelissier T, Ardid D, Hernandez A, Eschalier A. Evidence for a monoamine mediated, opioid-independent, antihyperalgesic effect of venlafaxine, a non-tricyclic antidepressant, in a neurogenic pain model in rats. Pain 2003; 103: 229–235.
23. Mico JA, Ardid D, Berrocoso E, Eschalier A. Antidepressants and pain. Trends Pharmacol Sci 2006; 27: 348–354.
24. Choucair-Jaafar N, Salvat E, Freund-Mercier MJ, Barrot M. The antiallodynic action of nortriptyline and terbutaline is mediated by beta(2) adrenoceptors and delta opioid receptors in the ob/ob model of diabetic polyneuropathy. Brain Res 2014; 1546: 18–26.
25. Cazzola M, Page CP, Rogliani P, Matera MG. beta2-agonist therapy in lung disease. Am J Respir Crit Care Med 2013; 187: 690–696.
26. Filliol D, Ghozland S, Chluba J, Martin M, Matthes HW, Simonin F, Befort K, Gaveriaux-Ruff C, Dierich A, LeMeur M, Valverde O, Maldonado R, Kieffer BL. Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. Nat Genet 2000; 25: 195–200.
27. Matthes HW, Maldonado R, Simonin F, Valverde O, Slowé S, Kitchen I, Befort K, Dierich A, Le Meur M, Dolle P, Tzavara E, Hanoune J, Roques BP, Kieffer BL. Loss of morphine-induced analgesia, reward effect and withdrawal symptoms in mice lacking the mu-opioid-receptor gene. Nature 1996; 383: 819–823.
28. Simonin F, Valverde O, Smadja C, Slowé S, Kitchen I, Dierich A, Le Meur M, Roques BP, Maldonado R, Kieffer BL. Disruption of the kappa-opioid receptor gene in mice enhances sensitivity to chemical visceral pain, impairs pharmacological actions of the selective kappa-agonist U-50,488H and attenuates morphine withdrawal. Embo J 1998; 17: 886–897.
29. Chruscincki AJ, Rohrer DK, Schauble E, Desai KH, Bernstein D, Kobilka BK. Targeted disruption of the beta2 adrenergic receptor gene. J Biol Chem 1999; 274: 16694–16700.
30. Yalcin I, Megat S, Barthas F, Waltisperger E, Kremer M, Salvat E, Barrot M. The sciatic nerve cuffing model of neuropathic pain in mice. J Vis Exp 2014; 89: 51608.
31. Barrot M. Tests and models of nociception and pain in rodents. Neuroscience 2012; 211: 39–50.
32. Celerier E, Rivat C, Jun Y, Laulin JP, Larcher A, Reynier P, Simonnet G. Long-lasting hyperalgesia induced by fentanyl in rats: preventive effect of ketamine. Anesthesiology 2000; 92: 465–472.
33. Nadal X, Banos JE, Kieffer BL, Maldonado R. Neuropathic pain is enhanced in delta-opioid receptor knockout mice. Eur J Neurosci 2006; 23: 830–834.
34. Kremer M, Yalcin I, Nexo L, Wurtz X, Ceredig RA, Daniel D, Hawkes RA, Salvat E, Barrot M. The antiallodynic action of pregabalin in neuropathic pain is independent from the opioid system. Mol Pain 2016; 12: 174480691663347.
35. Duman RS. Pathophysiology of depression: the concept of synaptic plasticity. Eur Psychiatry 2002; 17 (Suppl 3): 306–310.
36. Vialou V, Feng J, Robison AJ, Nestler EJ. Epigenetic mechanisms of depression and antidepressant action. Annu Rev Pharmacol Toxicol 2013; 53: 59–87.
37. Rantamaki T, Yalcin I. Antidepressant drug action—from rapid changes on network function to network rewiring. Prog Neuropsychopharmacol Biol Psychiatry 2016; 64: 285–292.
38. Ceredig RA, Pierre F, Doridot S, Alduntzin U, Hener P, Salvat E, Yalcin I, Gaveriaux-Ruff C, Barrot M, Massotte D. Peripheral delta opioid receptors mediate formoterol anti-allodynic effect in a mouse model of neuropathic pain. Front Mol Neurosci 2020; 12: 324.
39. Gaveriaux-Ruff C, Kieffer BL. Delta opioid receptor analgesia: recent contributions from pharmacology and molecular approaches. Behav Pharmacol 2011; 22: 405–414.
40. Kabli N, Cahill CM. Anti-allodynic effects of peripheral delta opioid receptors in neuropathic pain. Pain 2007; 127: 84–93.
41. Hervera A, Negrete R, Leanez S, Martin-Campos J, Pol O. The role of nitric oxide in the local antiallodynic and anti-hyperalgesic effects and expression of delta-opioid and cannabinoid-2 receptors during neuropathic pain in mice. J Pharmacol Exp Ther 2010; 334: 887–896.
42. Mika J, Przewlocki R, Przewlocka B. The role of delta-opioid receptor subtypes in neuropathic pain. Eur J Pharmacol 2001; 415: 31–37.
43. Saiioh A, Nagase H. Delta opioid receptor (DOR) ligands and pharmacology: development of indolo- and quinolino-morphinan derivatives based on the message-address concept. Handb Exp Pharmacol 2018; 247: 3–19.
44. Castany S, Carcole M, Leanez S, Pol O. The antinociceptive effects of a delta-opioid receptor agonist in mice with painful diabetic neuropathy: involvement of heme oxygenase 1. Neurosci Lett 2016; 614: 49–54.
45. Gallantin EL, Meert TF. A comparison of the antinociceptive and adverse effects of the mu-opioid agonist morphine and the delta-opioid agonist SNC80. Basic Clin Pharmacol Toxicol 2005; 97: 39–51.
46. Peppin JF, Raffa RB. Delta opioid agonists: a concise update on potential therapeutic applications. J Clin Pharm Ther 2015; 40: 155–166.
47. Binder W, Mousa SA, Sitte N, Kaiser M, Stein C, Schäfer M. Sympathetic activation triggers endogenous opioid
release and analgesia within peripheral inflamed tissue. Eur J Neurosci 2004; 20: 92–100.

48. Zhao X, Xu Y, Zhao Q, Chen CR, Liu AM, Huang ZL. Curcumin exerts antinociceptive effects in a mouse model of neuropathic pain: descending monoamine system and opioid receptors are differentially involved. Neuropharmacology 2012; 62: 843–854.

49. Mennicken F, Zhang J, Hoffert C, Ahmad S, Beaudet A, O’Donnell D. Phylogenetic changes in the expression of delta opioid receptors in spinal cord and dorsal root ganglia. J Comp Neurol 2003; 465: 349–360.

50. Busch-Dienstfertig M, Stein C. Opioid receptors and opioid peptide-producing leukocytes in inflammatory pain–basic and therapeutic aspects. Brain Behav Immun 2010; 24: 683–694.

51. Francois A, Low SA, Sypek EI, Christensen AJ, Sotoudeh C, Beier KT, Ramakrishnan C, Ritola KD, Sharif-Naeini R, Deisseroth K, Delp SL, Malenka RC, Luo L, Hantman AW, Scherrer G. A brainstem-spinal cord inhibitory circuit for mechanical pain modulation by GABA and enkephalins. Neuron 2017; 93: 822–839.e826.

52. Maruo K, Yamamoto H, Yamamoto S, Nagata T, Fujikawa H, Kanno T, Yaguchi T, Maruo S, Yoshiya S, Nishizaki T. Modulation of P2X receptors via adrenergic pathways in rat dorsal root ganglion neurons after sciatic nerve injury. Pain 2006; 120: 106–112.

53. Trimmer PA, Evans T, Smith MM, Harden TK, McCarthy KD. Combination of immunocytochemistry and radioligand receptor assay to identify beta-adrenergic receptor subtypes on astroglia in vitro. J Neurosci 1984; 4: 1598–1606.

54. Mantyh PW, Rogers SD, Allen CJ, Catton MD, Ghilardi JR, Levin LA, Maggio JE, Vigna SR. Beta 2-adrenergic receptors are expressed by glia in vivo in the normal and injured central nervous system in the rat, rabbit, and human. J Neurosci 1995; 15: 152–164.

55. Cao TT, Brelo A, von Zastrow M. The composition of the beta-2 adrenergic receptor oligomer affects its membrane trafficking after ligand-induced endocytosis. Mol Pharmacol 2005; 67: 288–297.

56. Jordan BA, Trapaide N, Gomes I, Nivarthi R, Devi LA. Oligomerization of opioid receptors with beta 2-adrenergic receptors: a role in trafficking and mitogen-activated protein kinase activation. Proc Natl Acad Sci USA 2001; 98: 343–348.

57. Ramsay D, Kellett E, McVey M, Rees S, Milligan G. Homo- and hetero-oligomeric interactions between G-protein-coupled receptors in living cells monitored by two variants of bioluminescence resonance energy transfer (BRET): hetero-oligomers between receptor subtypes form more efficiently than between less closely related sequences. Biochem J 2002; 365: 429–440.

58. Scherrer G, Imamachi N, Cao Y-Q, Contet C, Mennicken F, O’Donnell D, Kieffer BL, Basbaum AI. Dissociation of the opioid receptor mechanisms that control mechanical and heat pain. Cell 2009; 137: 1148–1159.