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DIFFERENTIAL NOXIOUS AND MOTOR TOLERANCE OF CHRONIC DELTA OPIOID RECEPTOR AGONISTS IN RODENTS

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Abstract

In the present study, we asked whether multiple intrathecal injections of deltorphin II, a selective delta opioid receptor (DOPR) agonist, induced DOPR tolerance in three behavioral assays. Unilateral inflammation caused by complete Freund's adjuvant (CFA) injection into the rat or mouse hind paw (CFA model) induced thermal hyperalgesic response that was transiently and dose-dependently reduced by intrathecal administration of deltorphin II or morphine. In both rodent species, the effect of deltorphin II was not modified by a single prior administration of deltorphin II, suggesting an absence of acute tolerance in this paradigm. Repeated administration of intrathecal deltorphin II or s.c. SB-235863 (five consecutive injections over 60 h) also failed to impair the antihyperalgesic response to delta opioid receptor agonist, whereas repeated intrathecal or s.c. injections of morphine induced a significant decrease in the subsequent thermal antihyperalgesic response to morphine. In mice, deltorphin II also induced a rapid, transient motor incoordination/ ataxia-like behavior as tested with the accelerating rotarod. In contrast to the antihyperalgesic responses, tolerance to the motoric effect of deltorphin II was evident in mice previously exposed to multiple intrathecal agonist injections, but not multiple saline administrations. Using the tail flick antinociceptive test, we found that DOPR-mediated analgesia was significantly reduced by repeated exposure to deltorphin II. Altogether, these observations suggest that repeated injections of DOPR agonists induce differential tolerance effects on antihyperalgesic, antinociceptive, and motor incoordination/ataxia-like behaviors related to DOPR activation by deltorphin II. © 2009 Published by Elsevier Ltd on behalf of IBRO.

Keywords

tolerance; delta opioid receptor; antihyperalgesia; locomotion; deltorphin; inflammation

Opioids are the most potent analgesics for treating moderate to severe pain. This class of medication, which includes morphine and its analogues, is also widely used for the treatment of persistent pain (Bodnar, 2007). However, clinicians concur that important side-effects associated to these drugs have limited their utility (McQuay, 1999). Among other constraints, development of analgesic tolerance is certainly the most problematic adverse effect of prolonged treatment with this class of drugs. Development of an effective

therapeutic alternative generating low tolerance risk for the treatment of chronic pain is consequently one of the biggest challenges for current pain research.

In addition to commonly prescribed opioids targeting the mu opioid receptor (MOPR), there is a growing interest in the delta opioid receptor (DOPR) agonists, mainly for their lack of typical opioid side-effects (i.e. nausea, respiratory depression, constipation, sedation) (Porreca et al., 1984; May et al., 1989; Dondio et al., 1997; Szeto et al., 1999; Brandt et al., 2001b; Petrillo et al., 2003; Gallantine and Meert, 2005). Indeed, drugs selectively activating DOPR were found to produce antinociception in various pain models. Interestingly, these drugs were also shown to exhibit enhanced potency in models of neuropathic and inflammatory pain (Desmeules et al., 1993; Stewart and Hammond, 1994; Fraser et al., 2000a; Hurley and Hammond, 2000; Qiu et al., 2000; Brandt et al., 2001a; Mika et al., 2001; Cahill et al., 2003; Petrillo et al., 2003; Gallantine and Meert, 2005). Moreover, prolonged treatment with DOPR agonists failed to induce analgesic tolerance in cancer and neuropathic pain conditions (Onofrio and Yaksh, 1983; Krames, 1986; Mika et al., 2001; Petrillo et al., 2003). To date, it is however unclear whether or not DOPR-selective agonists generate tolerance following chronic administration for the treatment of inflammatory pain.

DOPR is a member of the G protein-coupled receptor family (Evans et al., 1992; Kieffer et al., 1992). As with many other members of this family, activation of DOPR by an agonist leads, in various cellular models, to G protein uncoupling, desensitization, and receptor internalization (Varga, 2003; Varga et al., 2004). Although not completely understood, *in vivo* regulation of DOPR appears to be more intricate. Under certain conditions, levels of membrane-associated DOPR appear to be selectively increased in a subpopulation of spinal cord and ganglia neurons (Cahill et al., 2001, 2003; Kim and von Zastrow, 2003; Morinville et al., 2003; Patwardhan et al., 2005; Gendron et al., 2006), suggesting that trafficking of this receptor is tightly regulated. Recent evidence also suggests that desensitization and downregulation of DOPR are differentially controlled in various areas of the CNS (Jutkiewicz et al., 2005). In a model of peripheral inflammation, we also observed such a dichotomy. Indeed, we recently demonstrated that antihyperalgesic effects of the DOPR selective agonist deltorphin II were increased by sustained morphine treatment, while its antinociceptive properties were impaired (Gendron et al., 2007a).

The present study was designed to investigate the differential development of acute and chronic tolerance to delta opioid selective agonists. The effects of multiple intrathecal deltorphin II injections on hyperalgesia, nociception and motor functions were measured using the Hargreaves' plantar test, hot water immersion tail flick test and the accelerating rotarod, respectively.

EXPERIMENTAL PROCEDURES

Animals

Studies in rats—Adult male Sprague-Dawley rats (200–225 g; Charles-River, St-Constant, QC, Canada) were maintained on a 12-h light/dark cycle (6:00–18:00 h). Laboratory chow and water were available *ad libitum*. Studies were conducted between 7:00 and 11:00 (light cycle). Experiments were approved by the animal care committee of the Université de Sherbrooke in compliance with the policies and directives of the Canadian Council on Animal Care and guidelines from the International Association for the Study of Pain.

Studies in mice—In the second set of experiments, adult male C57BL/6 mice (19–26 g; Charles River, Wilmington, MA, USA) were used. Mice were housed in groups of four and maintained on a 12-h light/dark cycle (07:00–19:00 h). Laboratory chow and water were

available *ad libitum*. Studies were conducted between 7:00 and 11:00 (light cycle). Experiments were approved by the local animal care committee of the University of Washington and were in compliance with policies and directives of the Institutional Animal Care and Use Committees and guidelines from the International Association for the Study of Pain. Experiments were conducted in order to minimize the number of animals used and their suffering.

Induction of inflammation

Unilateral inflammation of the hind limb was induced by a single s.c. injection (100 μ l for rats and 50 μ l for mice) of complete Freund's adjuvant (CFA; Calbiochem, San Diego, CA, USA) in the plantar surface of the hind paw under brief isoflurane anesthesia (3%; 1 l/min). Behavioral testing was carried out 72 h after CFA injection as described below.

Morphine sulfate (lots 139138, 124895, 140684 and 143164; Sandoz, Boucherville, QC, Canada) was diluted in sterile saline solution to a concentration of 10 mg/ml and stored at room temperature until use. For behavioral testing, morphine was injected subcutaneously or intrathecally (i.t.) in rats.

Drugs

Deltorphan II (lots 074K11261 and 025K12731; Sigma, St. Louis, MO, USA), a DOPR-selective agonist, was dissolved in sterile saline solution (0.9% NaCl) to a concentration of 2 mg/ml and stored at -20°C in aliquots until use. For the experiments, deltorphan II was diluted in sterile saline solution and injected i.t. in a final volume of 5 μ l for mice, and 30 μ l for rats. Intrathecal injections were done free hand in nonanesthetized mice as described previously (Hylden et al., 1991; Fairbanks, 2003), whereas rats were injected while under light isoflurane anesthesia (3%; 1 l/min). Briefly, a 30 g $\frac{1}{2}$ -in. needle mounted on a Luer tip Hamilton syringe (VWR, Montréal, QC, Canada) was inserted into the L5–L6 intervertebral space (at the level of the cauda equina) and saline or deltorphan II injected over a 2 s period of time. Appropriate placement of the needle was confirmed by observing a brief twitch of the tail (both in rats and mice). To assess for deltorphan II-induced receptor tolerance, deltorphan II was injected repeatedly. Acute tolerance was tested by measuring the effect of a challenge dose of deltorphan II injected 2 h after either saline or deltorphan II. Chronic tolerance was evaluated by measuring the behavioral effect of various doses of appropriate drug in animals previously treated with five consecutive injections (one injection every 12 h) of either saline or drug. Behavioral testing was conducted exactly 12 h after the fifth injection. The chosen dose of deltorphan II corresponded to a nearly complete relief of ipsilateral thermal hyperalgesia and no contralateral latency modification (in concordance with our prior results (Gendron et al., 2007b).

SB-235863, a DOPR-selective agonist was synthesized as previously described (Petrillo et al., 2003). For further details, refer to supplemental material section. Compound was dissolved at a final concentration of 20 mg/ml in a saline solution containing 2-hydroxypropyl- β -cyclodextrin (HCD; 100 mg/ml; Sigma, St. Louis, MO, USA) and stored at 4°C until use. For behavioral testing, SB-235863 was injected subcutaneously in rats.

Behavioral testing

Plantar test—Development of hyperalgesia and antihyperalgesic effects of drugs was assessed using the plantar test (i.e. response to noxious heat stimulus). To test for thermal withdrawal thresholds, animals were first placed in Plexiglas enclosures set on a glass surface (for mice: IITC Life Science, Inc., Woodland Hills, CA, USA; for rats: Ugo Basile, Comerio VA, Italy) for a 15 min habituation period, 24 h prior to baseline measurements. The following day, the heat source was positioned under the plantar surface of the hind paw

after a 15 min habituation period and the latency for each hind paw withdrawal in response to radiant heat was measured three times in alternation (corresponding to -72 h). Afterward, CFA was injected in the hind paw as described above and baseline withdrawal latencies (thereafter identified as 0 min) were measured again 72 h later, prior to drug injection. Subsequently, latencies to paw withdrawal were recorded every 15 min over a period of 60 min. The intensity of the light beam was adjusted so that baseline latencies were approximately 10 s in naive animals. A cutoff time of 20 s was imposed to prevent tissue damage. If an animal reached the cutoff, the light beam was automatically turned off and the animal was assigned the maximum score. The maximum possible antihyperalgesic effect (MPAHE, return to baseline pre-injury withdrawal thresholds) of drugs in CFA-injected animals was calculated, for the inflamed hind paw (i.e. the paw injected with CFA), according to the following formula: $\% \text{ MPAHE} = 100 \times [(\text{test latency}) - (\text{baseline latency following CFA})] / [(\text{baseline prior to CFA}) - (\text{baseline latency following CFA})]$. From the latter calculation, 0% MPAHE represents no antihyperalgesic effect of the drug while a 100% MPAHE corresponds to a complete relief of the hyperalgesia, i.e. response latency to radiant heat identical to baseline prior to CFA injection.

Rotarod—Development of tolerance to motor incoordination/ ataxia-like behavior following i.t. injection of deltorphin II was evaluated by measuring the motor capacities of mice on the accelerating rotarod (Columbus Instruments, Columbus, OH, USA). In this test, we have previously observed that deltorphin II induces a DOPR-mediated dose-dependent decrease in motor functions characterized by hind limbs abduction and Straub tail (Gendron et al., 2007b). Mice were trained on a 3-day schedule including three 5-min sessions per day. The first four sessions were done in constant acceleration from 4 to 20 rpm over a 5-min period. In the following five sessions, the rotarod acceleration was set from 4 to 30 rpm over a 5-min period. During training sessions 1 through 6, mice were repositioned on the rotarod until they succeeded in remaining on the rotarod for 100 s. The latency to fall during the last training session was recorded and expressed as the mean performance before deltorphin II injection (in s). Each mouse received five saline (5 μ l) or deltorphin II (2.5 μ g) injections (one injection every 12 h). Twelve hours after the last injection (on day 3, 10–15 min after the last training), mice were injected i.t. with either saline solution (5 μ l) or deltorphin II (2.5 μ g) and tested at 5, 15, and 60 min post-injection. Performance was quantified as time spent on the rotarod (s).

Tail flick test—Development of antinociceptive tolerance (chronic) to deltorphin II was tested in mice repeatedly treated with saline (5 μ l) or deltorphin II (2.5 μ g). Each mouse received five saline or deltorphin II injections (one injection every 12 h). Twelve hours after the last injection, tail flick latencies (tail immersion in a 52.5 °C waterbath) following a challenge dose of deltorphin II (5 μ g) were measured (in s) every 10 min for a period of 60 min.

Calculations and statistical analysis

Calculations were done with Excel (2007), graphs with SigmaPlot 8.0, and statistical analysis with Prism GraphPad 5.0. Data are expressed as the mean $\pm$ SEM. *P*-values are presented in figure legends.

RESULTS

Unilateral inflammation: visual confirmation

Injection of CFA into the plantar surface of the right hind paw rapidly induced edema and swelling that persisted over at least 3 days (72 h), after which both rats and mice avoided bearing their body weight on their inflamed hind paw.

*Experiments performed in rats.***Antihyperalgesic potency of intrathecal deltorphin II and morphine**

As shown in Fig. 1A, intrathecal injection of deltorphin II induced a time- and dose-dependent alleviation of CFA-induced ipsilateral thermal hyperalgesia. For all effective doses of deltorphin II, the effect was maximal at 15 min after the injection and returned to baseline after 30–60 min. Intrathecal saline had no effect on the paw withdrawal latency. Intrathecal injection of morphine also induced a significant time- and dose-dependent antihyperalgesic effect and the effect peaked at 15 min after the injection (Fig. 1B). Deltorphin II did not affect the paw withdrawal latency in response to radiant heat applied to the contralateral hind paw while higher doses of morphine induced antinociception (not shown).

Acute analgesic tolerance to deltorphin II

Seventy-two hours after induction of inflammation, rats were i.t.-injected once with saline (30 μ l) or with deltorphin II (10 μ g). Two hours after the first injection, rats were challenged with 10 μ g of intrathecal deltorphin II and the latency to paw withdrawal in response to radiant heat was measured (Fig. 2). Regardless of the pretreatment, a subsequent 10 μ g-dose of intrathecal deltorphin II induced similar antihyperalgesic responses (amplitude and kinetic) in both groups. Therefore, there was no evidence for acute DOPR tolerance under these conditions.

Chronic analgesic tolerance to deltorphin II and morphine

To assess the development of analgesic tolerance following repeated agonist treatment, rats treated with CFA were injected every 12 h with the vehicle or the agonist for a total of six injections. The last injection was given exactly 72 h after induction of inflammation. As shown in Fig. 3A and C, repeated intrathecal injections of deltorphin II (10 μ g every 12 h) did not significantly modify its analgesic potency in the CFA model of inflammation. Indeed, the MPAHE (% MPAHE) of deltorphin II, as calculated at 15 min post-injection, revealed no difference between saline and deltorphin II-pretreated groups (Fig. 3C). In contrast, intrathecal morphine pretreatment (3 μ g once every 12 h) induced a significant decrease in the antihyperalgesic potency of morphine (Fig. 3B, D). Interestingly, neither deltorphin (II) nor morphine-repeated injections significantly prevented the development of hyperalgesia induced by inflammation (Fig. 3; comparing baseline latencies to paw withdrawal at time 0 min for chronic saline, chronic deltorphin II and chronic morphine groups).

Analgesic tolerance to systemically-injected SB-235863, a nonpeptidic DOPR-selective agonist, and morphine

To see whether systemically-injected drugs would induce analgesic tolerance, the peripherally active and brain penetrant DOPR agonist SB-235863 was then tested in the CFA model of inflammation. As shown in Fig. 4A., s.c. SB-235863 at 30 mg/kg induced time-dependent antihyperalgesia with maximal effect 30 min after drug injection. As opposed to intrathecal deltorphin II, s.c. SB-235863 failed to completely relieve the inflammation-induced thermal hyperalgesia. Indeed, at higher dose (71 mg/kg) the antihyperalgesic effect induced by SB-235863 was not different from that produced by 30 mg/kg (not shown). However, at such a high dose SB-235863 was found to induce hemolysis-like effects (severe site dermatitis and hematuria) as early as 1 h after its administration. This effect was not seen with the 30 mg/kg dose even after repeated injections. Repeated s.c. injections of SB-235863 (30 mg/kg) did not modify its analgesic effect in the CFA model of inflammation (Fig. 4A). In contrast, s.c. morphine pretreatment

(3 mg/kg once every 12 h) induced a significant decrease in the antihyperalgesic potency of morphine (Fig. 4B). Comparing the % MPAHE for acute and chronic treatment with DOPR agonists shows no modification of their analgesic potency following intrathecal (106.0±27.7% vs. 85.5%±7.4% MPAHE respectively for one or repeated deltorphin injections) or s.c. (51.2±14.1% vs. 64.1%±14.8% MPAHE for one or repeated SB-235863 injections) injections. By contrast, repeated injections of morphine induced a significant decrease in its analgesic potency for both intrathecal (from 160.3±21.1% to 87.2±8.39% MPAHE) and s.c. injections (from 124.3±27.7% vs. 42.4%±13.4% MPAHE) (Fig. 4C). Nevertheless, morphine is still active after chronic pretreatment, but its efficiency is greatly diminished.

Experiments performed in mice—In mice, we have recently shown that intrathecal deltorphin II induced a dose-related motor incoordination/ataxia-like behavior as well as transient Straub tail symptoms (Gendron et al., 2007b). Because these effects were barely evident in rats at the doses required to produce analgesia, we designed a set of experiments aiming at evaluating the appearance of tolerance to deltorphin II in various behaviors. Although these motor incoordination/ataxia-like behaviors do not impair the perception and the ability of mice to respond (i.e. paw withdrawal) to noxious heat stimuli (Gendron et al., 2007b), repeated deltorphin II injections have the tendency to increase movements (very clear in mice) and activity (our unpublished observations and Spina et al., 1998; Fraser et al., 2000b). To avoid the possibility that motoric effects could somehow interfere with our ability to measure paw withdrawal after chronic treatments, we decided to induce tolerance with a 2.5 µg dose but to test analgesia with a smaller dose (i.e. 1 µg).

Analgesic tolerance to deltorphin II

Development of antihyperalgesic tolerance to deltorphin II was tested in mice injected with CFA in the plantar surface of the right hind paw. Acute tolerance was evaluated in CFA-treated mice i.t.-challenged with 1.0 µg of deltorphin II (submaximal dose ≈ED₅₀) 2 h after a first injection of saline, 2.5 or 10 µg of deltorphin II (Fig. 5A). No acute antihyperalgesic tolerance was evident. Development of chronic tolerance was also tested in mice treated every 12 h with saline (5 µl intrathecal) or deltorphin II (2.5 µg; intrathecal) with the first administration beginning 12 h following CFA injection and continuing until 72 h after induction of inflammation. The 2.5 µg-dose of deltorphin II for the pretreatment was chosen for its nearly complete relief of thermal hyperalgesia in this test without affecting the withdrawal latency of the contralateral hind paw (Gendron et al., 2007b). As shown in Fig. 5B, repeated intrathecal injections of deltorphin II did not induce antihyperalgesic tolerance.

Tolerance to motoric effects of deltorphin II

To assess whether tolerance to the motor incoordinating effects was evident, we challenged the mice with deltorphin II and measured their subsequent performance on the accelerating rotarod. In mice repeatedly treated with intrathecal saline, deltorphin II markedly decreased the time spent on the rotarod, 5 and 15 min after the injection (Fig. 6, respectively 58±17 and 101±23 s as compared to 192±21 s before deltorphin II injection). By contrast, mice chronically treated with the agonist prior to the challenge were found to be insensitive to further administration of deltorphin II (Fig. 6). Following repeated administration of 2.5 µg deltorphin II, a challenge dose did not significantly affect the time spent on the rotarod (178±19 and 215±20 s respectively for 5 and 15 min) when compared to the performance before the injection (192±21 s). Note that the effect of multiple injections of deltorphin II on motor functions seemed to have a similar pattern for mice injected with CFA. However, because of paw swelling and edema, motor functions of these animals were not assayed with the rotarod. Although the incoordination/ataxia-like symptoms were weaker after repeated deltorphin II injections, Straub tail was not completely abolished (data not shown).

Tolerance to antinociceptive effects of deltorphin II

Development of analgesic tolerance to deltorphin II was further assessed using the immersion tail flick test. Mice were repeatedly treated with either intrathecal saline (5 μ l) or deltorphin II (2.5 μ g). As shown in Fig. 7, for both groups, deltorphin II (5 μ g) induced a transient increase in the latency to tail withdrawal. This antinociceptive effect of deltorphin II peaked 10 min after the injection and response latencies returned to baseline levels within 30–40 min. However, the deltorphin II-induced increase in the latency to tail withdrawal was significantly lower in mice repeatedly treated with deltorphin II than the response of mice pretreated with repeated saline injections. Note that in the tail flick test, the dose of deltorphin II used was determined based on a pilot study indicating that at least 5 μ g was required to induce a significant antinociceptive response in naive animals. At this dose, deltorphin's antinociceptive effect in this test was previously shown to be unchanged in MOPR-KO mice (Hosohata et al., 2000) and blocked by DOPR antisense oligonucleotides (Tseng et al., 1994), providing evidence for a selective action of deltorphin II on DOPR in this test.

DISCUSSION

In the present study, we investigated the effects of multiple injections of DOPR-selective agonists (deltorphin II and SB-235863) using various treatment and assessment paradigms. In a model of CFA-induced inflammation, we found a lack of both acute and chronic tolerance to delta agonists' antihyperalgesic effect. By contrast, when DOPR-mediated motor incoordination/ataxia-like and antinociception (acute pain) behaviors were assessed, repeated injection of deltorphin II was found to reduce DOPR-mediated response and to induce the development of tolerance. Interestingly, both systemic and intrathecal morphine was found to induce antihyperalgesic tolerance.

Although morphine and other MOPR-activating drugs are still considered as the most potent analgesics (Bodnar, 2007), an increasing focus is being directed towards DOPR-selective agonists. In fact, these ligands were shown to possess significant analgesic properties in diverse pain paradigms (Bilsky et al., 1995; Chiba et al., 1996; Hosohata et al., 2000; Baker et al., 2002). Indeed, compounds activating DOPR relieve inflammation-induced thermal hyperalgesia (Desmeules et al., 1993; Stewart and Hammond, 1994; Fraser et al., 2000a; Hurley and Hammond, 2000; Qiu et al., 2000; Brandt et al., 2001a; Cahill et al., 2003; Petrillo et al., 2003; Gallantine and Meert, 2005; Gendron et al., 2007a; Le Bourdonnec et al., 2008; Gaveriaux-Ruff et al., 2008; Hernandez et al., 2009; Codd et al., 2009), neuropathic pain (Desmeules et al., 1993; Mika et al., 2001; Petrillo et al., 2003; Holdridge and Cahill, 2007; Kabli and Cahill, 2007), and cancer-related pain (Brainin-Mattos et al., 2006). As confirmed in the present study, spinal delivery of deltorphin II induces a time- and dose-dependent antihyperalgesic effect both in rats and mice (see Gendron et al., 2007b for the dose-response curves of deltorphin II in mice), an effect previously shown to be DOPR-mediated (Gendron et al., 2007b).

One of the most challenging problems with MOPR agonists refers to the development of tolerance appearing during prolonged treatments. Particularly, it is well known that repeated injections of morphine induce analgesic tolerance in various inflammatory pain models such as in formalin- (Rahman et al., 1994) or carrageenan-induced inflammation (Le Guen et al., 1998). In the present study, we report that repeated systemic and intrathecal injections of morphine decrease its analgesic efficacy in the CFA model of inflammation. In line with our observations, others have previously noticed such a decrease in morphine's analgesic potency in the CFA model of inflammation (Li et al., 1999; Liang et al., 2006; Fernandez-Duenas et al., 2007), at least following systemic administration. By contrast, Zollner et al. (2008) recently observed that chronic use of morphine does not induce analgesic tolerance.

The major difference between this study and our findings relies on the fact that, on the day of their experiment, Zollner et al. administered morphine locally (i.e. intraplantar), therefore revealing absence of peripheral tolerance to repeated systemic injections. Our results rather suggest an onset of central tolerance to chronic morphine treatment.

If others reported tolerance to repeated systemic morphine injections in the CFA model of inflammation (Li et al., 1999; Liang et al., 2006; Fernandez-Duenas et al., 2007), to our knowledge we are the first to demonstrate morphine tolerance following spinal delivery. In humans, analgesic tolerance to prolonged intrathecal morphine was observed in patients suffering from cancer-related pain (Onofrio and Yaksh, 1983; Krames et al., 1986). Most importantly, these studies also showed a prolonged analgesic effect of i.t. delivered DADLE, an effect possibly mediated by DOPR (Onofrio and Yaksh, 1983; Krames et al., 1986). Although one could argue that cancer pain and inflammatory pain are different, cancer pain is known to have an inflammatory component (Honore et al., 2000).

In vivo, it is not clear whether or not development of antihyperalgesic tolerance to DOPR agonists during treatment of inflammatory pain occurs. A major finding of the present study was that various doses of intrathecal deltorphin II failed to decrease the antihyperalgesic effects of a subsequent stimulation of spinal DOPR in CFA animals. Indeed, we showed a constant analgesic potency of i.t.-administered DOPR agonists even after five consecutive injections of deltorphin II (two injections per day). These observations provide the first evidence that antihyperalgesia induced by intrathecal deltorphin II does not result in acute or chronic tolerance in a model of inflammation.

To see whether systemic injection of DOPR agonists induces tolerance, we used a nonpeptidic DOPR-selective agonist derived from codeine, SB-235863. This compound was chosen because of its high selectivity for DOPR (Dondio, 2000; Dondio et al., 2001; Petrillo et al., 2003), because it is devoid of pro-convulsing activity, because it crosses the blood–brain barrier (Petrillo et al., 2003), and because it has a chemistry similar to that of morphine. Interestingly, while as potent as morphine at reducing carrageenan-induced hyperalgesia, this compound was not found to induce referenced side-effects often associated with morphine (Dondio, 2000; Dondio et al., 2001; Petrillo et al., 2003). In our hands, SB-235863 failed at completely relieving inflammation-induced thermal hyperalgesia. This discrepancy could be explained by various experimental differences such as diverse routes of administration, different models of inflammation, or the nature of the vehicle used. In the present study, we observed that SB-235863 retains its analgesic properties when repeatedly administered subcutaneously to inflamed rats. These observations demonstrate that antihyperalgesic tolerance did not develop during a prolonged treatment of inflammatory pain with a second DOPR-selective agonist having a nonpeptidic structure and different route of administration. Consistent with our findings, others have also shown absence of analgesic tolerance following repeated oral administrations of SB-235863 in animal models of carrageenan-induced inflammation and partial sciatic nerve ligation-induced neuropathy (Petrillo et al., 2003).

Previous studies, including ours, have shown similar effects of intrathecal deltorphin II in rats and in mice. However, we recently revealed that intrathecal deltorphin II induces rapid and transient motor incoordination/ataxia-like behaviors in mice, an effect barely seen in rats. To see whether tolerance to deltorphin II might differently developed in various behaviors, we therefore used mice to measure the effects of deltorphin II in different paradigms.

A number of groups have already reported antinociceptive tolerance following repeated exposure to DOPR-selective agonists (Bhargava and Zhao, 1996; Tseng et al., 1997; Zhao

and Bhargava, 1997; Broom et al., 2002). Using the hot water immersion tail flick test, we first confirmed that single intrathecal injection of deltorphin II induced a transient antinociceptive effect in mice. Interestingly, the dose of intrathecal deltorphin II required to produce analgesia in this paradigm was significantly higher than in the CFA model, probably because membrane expression of DOPR in spinal cord neurons of naive animals is very low (Cahill et al., 2001; Gendron et al., 2007a). In accordance with previous reports (Kest et al., 1994; Bhargava and Zhao, 1996; Zhao and Bhargava, 1997), in this pain paradigm chronic treatment with deltorphin II induced a rapid development of antinociceptive tolerance. These results therefore reveal the existence of a dichotomy within analgesic functions of DOPR, i.e. between its antihyperalgesic and antinociceptive effects.

The concept that diverse DOPR-mediated behaviors differently raise tolerance is intriguing. Our observations effectively suggest that sustained use of DOPR-selective agonists produces persistent antihyperalgesia while antinociception is lost. In rodents, besides a reduction of antinociceptive properties of DOPR-selective agonists following multiple injections (present study and Tseng et al., 1997; Zhao and Bhargava, 1997; Broom et al., 2002), pro-convulsing effects of DOPR agonists, such as SNC-80, were also decreased (Comer et al., 1993; Broom et al., 2002; Jutkiewicz et al., 2005). Interestingly, Jutkiewicz et al. (2005) have shown a conserved antidepressant-like effect induced by SNC-80 in these animals, therefore suggesting the existence of differential behavioral tolerance to delta opioid agonists. Taken together, these observations led us hypothesize a decrease in side-effects while antihyperalgesic and antidepressant-like properties would be preserved following prolonged administration of DOPR-selective agonists.

While performing the experiments described in the present study, a rapid loss of deltorphin II-induced motoric effects in mice was observed following repeated injections of this DOPR-agonist. This was confirmed by measuring the effect of intrathecal deltorphin II on rotarod performance indicating that development of motor tolerance also rapidly appears in this paradigm. This effect was also observed in a study performed by Jutkiewicz and coworkers (2005) showing tolerance to motoric effects induced by SNC-80 following repeated administrations.

Although the results presented in this study suggest the existence of opposing regulatory mechanisms of tolerance, the experiments were not designed to resolve this issue. Alternatively, absence of tolerance to DOPR agonists in regard to treatment of persistent pain (present study) and anxiety/depression (Jutkiewicz et al., 2005) (as opposed to antinociceptive, motoric, and pro-convulsive effects) could be due to a tonic neuronal activity present in these circuits. Indeed, blockade or absence of DOPR was shown to affect the hedonic state of anxiety and depression (Filliol et al., 2000; Saitoh et al., 2004, 2005), but also the level of neuropathic pain-induced allodynia (Nadal et al., 2006) and the development of inflammation-induced hyperalgesia (Gaveriaux-Ruff et al., 2008). Since we have shown that antihyperalgesic, but not locomotor effect, of deltorphin II was lost in MOPR-KO mice (Gendron et al., 2007b), the hypothesis that differential development of tolerance could be related to MOPR or MOPR/DOPR interaction should not be ruled out.

CONCLUSION

In conclusion, the present study proves the existence of a dichotomy between development of tolerance regarding diverse behavioral effects mediated by DOPR activation. In particular, we demonstrated a lack of antihyperalgesic tolerance to delta-selective agonists in a model of peripheral inflammation induced by injection of CFA into the plantar surface of the paw. As opposed to its antihyperalgesic effect, development of tolerance to deltorphin II was observed when its antinociceptive properties and motoric effects were measured. Rapid

loss of the motoric functions, combined with persistent antihyperalgesia induced by deltorphin II, implies major therapeutic clinical relevance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CFA	complete Freund's adjuvant
DOPR	delta opioid receptor
HCD	2-hydroxypropyl- β -cyclodextrin
i.t.	intrathecally
MOPR	mu opioid receptor
MPAHE	maximum possible antihyperalgesic effect

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APPENDIX

Supplementary data

Supplementary data associated with this article can be found, in the online version, at [doi: 10.1016/j.neuroscience.2009.03.053](https://doi.org/10.1016/j.neuroscience.2009.03.053).

Fig. 1. Antihyperalgesic effect of intrathecal deltorphin II and morphine in Sprague-Dawley rats. Sprague-Dawley rats were injected with CFA in the plantar surface of the hind paw. Seventy-two hours after CFA injection, the latency to paw withdrawal (in s) was tested every 15 min (from 0 to 60 min) after agonist injection using the Hargreaves test. (A) I.t.-administered deltorphin II (1, 3, 10 µg) induced a dose-dependent relief of thermal hyperalgesia (ipsilateral hind paw) ($F_{\text{Treatment}} = 17.48$ with $P < 0.0001$ two-way ANOVA followed by Bonferroni post hoc test). No effect of deltorphin II was observed on the contralateral hind paw (not shown). (B) I.t.-administered morphine (0.3, 1, 3 µg) induced a dose-dependent relief of thermal hyperalgesia (ipsilateral hind paw) ($F_{\text{Treatment}} = 36.26$ with

$P < 0.0001$, two-way ANOVA followed by Bonferroni post hoc test). No effect was seen on the contralateral hind paw (not shown). Results presented for saline-treated rats are the same as shown in (A); -72 h indicates the baseline for the latency to paw withdrawal before CFA injection. Number given in the legend inset represents the number of animals per group.

Fig. 2.

Acute tolerance to deltorphin II-induced antihyperalgesia in rats. Sprague–Dawley rats were injected with CFA in the plantar surface of the hind paw. Seventy-two hours after CFA injection, paw withdrawal latencies (in s) to noxious heat (plantar test) were recorded every 15 min (from 0 to 60 min) following an intrathecal challenge of deltorphin II (10 µg) in rats previously treated 2 h before with either saline (●; 30 µl) or deltorphin II (○; 10 µg). When rats were challenged, no statistical difference occurred in latencies to paw withdrawal between groups ($F_{\text{Treatment}}=2.561$ with $P=0.1150$, two-way ANOVA followed by Bonferroni post hoc test). Number given in the legend inset represents the number of animals per group.

Fig. 3.

Chronic tolerance to deltorphin II and morphine-induced antihyperalgesia in Sprague-Dawley rats. Sprague-Dawley rats were injected with CFA in the plantar surface of the hind paw. Seventy-two hours after CFA injection, paw withdrawal latencies (in s) to noxious heat (plantar test) were recorded every 15 min (from 0 to 60 min) following agonist administration. (A) Chronic antihyperalgesic tolerance to intrathecal deltorphin II was assessed in rats previously treated i.t. with five consecutive injections of saline (●; 30 μ l) or deltorphin II (○; 10 μ g). When rats were then challenged with deltorphin II (10 μ g), no statistical difference occurred for paw withdrawal latencies between groups ($F_{\text{Treatment}}=0.0056$ with $P=0.9405$, two-way ANOVA followed by Bonferroni post hoc test). (B) Chronic antihyperalgesic tolerance to intrathecal morphine was assessed in rats previously treated i.t. with five consecutive injections of saline (● 30 μ l) or morphine (○; 3 μ g). Repeated morphine injections strongly reduce its analgesic efficacy ($F_{\text{Treatment}}=39.75$ with $P=0.0028$, two-way ANOVA followed by Bonferroni post hoc test). ** $P<0.01$ when chronic saline and chronic morphine groups were compared. (C) Following pre-treatment with saline (●) or deltorphin II (○), the antihyperalgesic effect of increasing doses of intrathecal deltorphin II was expressed as the % MPAHE and there is no statistical

difference between groups ($F_{\text{Treatment}}=0.88$ with $P=0.3527$, two-way ANOVA followed by Bonferroni post hoc test). (D) Following intrathecal pre-treatment with saline (●) or morphine (○), the antihyperalgesic effect of increasing doses of intrathecal morphine was expressed as the % MPAHE and a significant decrease in morphine analgesic potency was obtained ($F_{\text{Treatment}}=29.67$ with $P<0.0001$, two-way ANOVA followed by Bonferroni post hoc test). ** $P<0.01$ and *** $P<0.001$ when chronic saline and chronic morphine groups were compared. Number given in the legend inset represents the number of animals per group.

Fig. 4. Chronic tolerance to SB-235863-induced antihyperalgesia in Sprague-Dawley rats. (A) Sprague-Dawley rats were injected with CFA in the plantar surface of the hind paw and received vehicle (HCD) once (●), repeated injections of HCD followed by a unique dose of SB-235863 (○) or repeated injections of SB-235863 (●). Seventy-two hours after CFA injection, paw withdrawal latencies (in s) to noxious heat (plantar test) were recorded every 15 min (from 0 to 60 min) following SB-235863 administration. SB-235863 partially, but significantly reverse CFA-induced thermal hyperalgesia ($F_{\text{Treatment}}=6.986$ with $P=0.0015$ two-way ANOVA followed by Bonferroni post hoc test). * $P<0.05$ and ** $P<0.01$ when SB-235863 groups were compared to vehicle group. When compared together, no difference

was observed between rats pre-treated or not with SB-235863 ($F_{\text{Treatment}}=0.7474$ with $P=0.3903$ two-way ANOVA followed by Bonferroni post hoc test). Number given in the legend inset represents the number of animals per group. (B) Chronic antihyperalgesic tolerance to s.c. morphine was assessed in rats previously treated subcutaneously with five consecutive injections of saline (●) or morphine (○; 3 mg/kg). Repeated morphine injections strongly reduce its analgesic efficacy ($F_{\text{Treatment}}=43.41$ with $P=0.0095$, two-way ANOVA followed by Bonferroni post hoc test). * $P<0.05$ when chronic saline and chronic morphine groups were compared. Number given in the legend inset represents the number of animals per group. (C) Comparative effect of drugs in rats pre-treated or not with repeated injections of the same drug. Chronic injections of morphine significantly reduced its antihyperalgesic effect ($P=0.0092$ and 0.0287 for intrathecal and s.c. injections respectively, with unpaired t -test), whereas DOPR selective agonists' repeated injections did not modify their analgesic potency ($P=0.6015$ and 0.5457 for deltorphin II and SB-235863 respectively, with unpaired t -test).

Fig. 5.

Acute and chronic tolerance to deltorphin II-induced antihyper-algesia in C57BL/6 mice. C57BL/6 mice were injected with CFA in the plantar surface of the hind paw. Seventy-two hours after CFA injection, paw withdrawal latencies (in s) to noxious heat (plantar test) were recorded every 15 min (from 0 to 60 min) following intrathecal administration of deltorphin II (1.0 µg) in mice previously treated with either saline or deltorphin II. (A) Acute tolerance to deltorphin II was tested in mice i.t.-injected 2 h earlier with saline (5 µl) or deltorphin II (2.5 or 10 µg). When mice were then challenged with 1.0 µg of deltorphin II, no difference was observed among any group ($F_{\text{Treatment}}=1.180$ with $P=0.3136$, two-way ANOVA followed by Bonferroni post hoc test). (B) Chronic antihyperalgesic tolerance to intrathecal

deltorphan II was assessed in mice previously treated i.t. with 5 consecutive injections of saline (●; 5 μ l) or deltorphan II (○; 2.5 μ g). No difference was observed among groups ($F_{\text{Treatment}}=0.5042$ with $P=0.4799$, two-way ANOVA followed by Bonferroni post hoc test). Number given in the legend inset represents the number of animals per group.

Fig. 6. Development of tolerance to deltorphin II-induced motor inco-ordination/ataxia-like behavior in C57BL/6 mice. C57BL/6 mice were i.t.-injected with saline or deltorphin II (2.5 μ g). The effect of deltorphin II on rotarod performances was evaluated in chronic saline (●) and chronic deltorphin (○) groups following deltorphin II challenge (2.5 μ g; intrathecal). Mice chronically treated with deltorphin II were insensitive to the motoric effect of a 6th injection ($F_{\text{Treatment}}=31.66$ with $P<0.001$, two-way ANOVA followed by Bonferroni post hoc test). ** $P<0.01$ when chronic saline and chronic deltorphin groups were compared. Number given in the legend inset represents the number of animals per group.

Fig. 7.

Acute and chronic tolerance to deltorphin II-induced antinoci-ception in C57BL/6 mice. C57BL/6 mice were i.t.-injected with saline or deltorphin II (2.5 µg). The effect of deltorphin II on tail withdrawal latencies was evaluated in chronic saline (●) and chronic deltorphin (○) groups following deltorphin II challenge (5 µg; intrathecal). Tail flick withdrawal latencies (in s) to noxious heat (tail immersion in 52.5 °C waterbath) were recorded every 10 min (from 0 to 60 min) following intrathecal administration of deltorphin II. Chronic deltorphin II injections significantly reduce antinociception produced by a 6th injection ($F_{\text{Treatment}}=8.995$ with $P=0.0035$, two-way ANOVA followed by Bonferroni post hoc test). * $P<0.05$ and ** $P<0.01$ when chronic saline and chronic deltorphin groups were compared. Number given in the legend inset represents the number of animals per group.