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Modulation of Morphine-induced Antinociception in Acute and Chronic Opioid Treatment by Ibudilast

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Background: **Opioid analgesics are effective in relieving chronic pain, but they have serious adverse effects, including development of tolerance and dependence. Ibudilast, an inhibitor of glial activation and cyclic nucleotide phosphodiesterases, has shown potential in the treatment of neuropathic pain and opioid withdrawal. Because glial cell activation could also be involved in the development of opioid tolerance in rats, the authors studied the antinociceptive effects of ibudilast and morphine in different models of coadministration.**

Methods: **Antinociception was assessed using male Sprague-Dawley rats in hot plate and tail-flick tests. The effects of ibudilast on acute morphine-induced antinociception, induction of morphine tolerance, and established morphine tolerance were studied.**

Results: **Systemic ibudilast produced modest dose-related antinociception and decreased locomotor activity at the studied doses of 2.5–22.5 mg/kg. The highest tested dose of 22.5 mg/kg produced 52% of the maximum possible effect in the tail-flick test. It had an additive antinociceptive effect when combined with systemic morphine. Coadministration of ibudilast with morphine did not attenuate the development of morphine tolerance. However, in morphine-tolerant rats, ibudilast partly restored morphine-induced antinociception.**

Conclusions: **Ibudilast produces modest antinociception, and it is effective in restoring but not in preventing morphine tolerance. The mechanisms of the effects of ibudilast should be better understood before it is considered for clinical use.**

THE treatment of acute and chronic severe pain remains a major challenge. Opioids, such as morphine and oxycodone, are used as primary analgesics in moderate to severe pain.[1,2] However, especially long-term opioid treatment has several problems, such as development of tolerance and dependence with withdrawal symptoms. Opioids can also cause respiratory depression, nausea, and constipation.[3] The adverse effects and tolerance with hyperalgesia may necessitate discontinuation of opioid treatment and result in inadequate pain control.

The classic models of explaining the development of opioid tolerance have mostly focused on changes in neurons themselves. The changes caused by repeated administration of opioids and consequent receptor activation may involve adaptive changes in the neurons, such as internalization of opioid receptors,[4] up-regulation of *N*-methyl-D-aspartate receptor function,[5,6] production of nitric oxide, or down-regulation of glutamate transporters.[7] Also, a counter-regulatory antiopioid system could be involved, including neuromodulators such as cholecystokinin or dynorphin.[8]

Recent studies suggest that opioid administration induces tolerance also *via* mechanisms other than those involving neurons. The glial cells of the central nervous system have previously been considered neuroimmune cells that mainly provide support and nutrition for the neurons. New data indicate that activated glial cells may modulate the activity of the nociceptive neurons in the central nervous system[9–11] and actively oppose the analgesic action of opioids on pain-transmission neurons. Particularly after repeated administration of opioids, various proinflammatory neuromodulatory substances, such as substance P, fractalkine, nitric oxide, interleukin (IL)-1, and tumor necrosis factor α, are released in the central nervous system.[12,13] These pronociceptive substances may attenuate opioid analgesia. The role of glial activation in this process still needs to be clarified. However, glial activation has also been suggested to be involved in the pathophysiology of neuropathic pain,[14,15] which is less responsive to opioids than nociceptive pain.

Glia have been reported to have an important role in modulating analgesia induced by chronic opioids.[9,10,12,16,17] One approach to target opioid tolerance is to depress the activation of glial cells. Ibudilast (AV411), a phosphodiesterase inhibitor in clinical use for asthma in Asia, has been shown to suppress glial activation. It suppresses lipopolysaccharide-induced production of inflammatory mediators such as tumor necrosis factor α, nitric oxide, IL-1, and IL-6 and increases the production of antiinflammatory cytokines, such as IL-10 by glia *in vitro*.[18–21] Recently, ibudilast was also shown to attenuate opioid-induced glial proinflammatory responses in rats.[22] The target protein for this effect of ibudilast on glial cells is not known. However, phosphodiesterase inhibition may also contribute to the antiinflammatory effects of ibudilast.[19] In addition, ibudilast is a weak adenosine receptor antagonist, but other target proteins have not been identified.[21,23] Ibudilast has a good safety profile, and therefore it is a good drug candidate that should be tested in opioid analgesia.

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Ibudilast was recently shown to increase the antinociceptive action of morphine in the tail-flick test and to reduce withdrawal symptoms.[22,23] It also attenuated chronic constriction injury–induced allodynia and was suggested to affect development of opioid tolerance.[21] However, the effect of ibudilast on opioid-induced antinociception and development of opioid tolerance has not been systematically investigated. Therefore, we tested the hypothesis that ibudilast could modify the antinociceptive effect of morphine in opioid-tolerant rats using the tail-flick and hot plate tests. We studied the antinociceptive effects of ibudilast alone and in coadministration of morphine. The focus was particularly on the effects of ibudilast in both the prevention and the reversal of morphine tolerance.

Materials and Methods

Animals

The research was conducted according to the guidelines of local authorities and the International Association for the Study of Pain.[24] The provincial government of Southern Finland approved the study protocol (Uudenmaan lääninhallitus, Helsinki, Finland).

We used male Sprague-Dawley rats (Harlan, Horst, The Netherlands; n = 6 or 7 per protocol; weight, 180–290 g). The rats were housed in clear plastic cages in temperature- and light-controlled rooms (23° ± 2°C; lights on at 7:00 AM, off at 7:00 PM). Tap water and standard rodent chow were available *ad libitum*. The behavioral tests were performed between 9:00 AM and 3:00 PM. Before the tests, the animals were habituated to the testing environment 60 min/day for 3 days. The same experimental animals were used in experiments I, IIIA, and IIIB. Between experiments I and IIIA, a 5-day washout period was used. After completion of the experiments, the animals were killed by decapitation.

Drugs

Morphine hydrochloride was purchased from the University Pharmacy (Helsinki, Finland) and ibudilast from APAC Pharmaceutical, LLC (Columbia, MD). Morphine was dissolved in physiologic saline (Sigma-Aldrich, St. Louis, MO). Ibudilast was first dissolved in polysorbate 20 (Tween® 20; Fluka Chemika, Buchs, Switzerland), and the mixture was diluted to 2% with physiologic saline. Saline or 2% polysorbate 20 dissolved in saline was used as control when appropriate. All morphine injections were administered subcutaneously (injection volume 2 ml/kg), and all ibudilast injections were administered intraperitoneally (injection volume 10 ml/kg). In the cotreatment experiments, ibudilast was administered at the same time as morphine. The drugs were administered in a randomized order, and the person who tested the animals was blinded to the treatment.

Nociceptive Tests

Tail-flick latencies were tested with a Ugo Basile 37360 (Comerio, Italy) tail-flick apparatus. In the test, the rats were restrained in hard plastic tubes covered with a dark cloth. The tests were repeated thrice at each time point using a 15-s interval, and the mean of these three values was used as the result. The infrared light was directed in turn at three different points of the middle third of the tail. The intensity was adjusted to produce a baseline latency of approximately 3.5 s. To avoid tissue damage, the cutoff was set at 10 s.

Hot plate tests were performed with a Harvard Apparatus Ltd. hot plate apparatus (Edenbridge, Kent, United Kingdom). In the test, the rats were kept inside a circular transparent plastic cage on the hot plate (52° ± 0.2°C). Licking or shaking the hind paw or jumping was considered as a sign of thermal nociception. Time to the first reaction was measured. To avoid tissue damage, the cutoff time was set to 60 s.

In all experiments, antinociception was assessed 30 and 120 min after administration of the test drugs. At each time point, the tail-flick test was performed first and the hot plate test was performed 20 s after that. The predrug (baseline) latencies were measured separately for each experiment day immediately before the administration of any drugs.

Motor Coordination and Spontaneous Locomotor Activity Evaluation

A rotarod apparatus (Palmer electric recording drum; United Kingdom; diameter, 80 mm; speed, 27 rpm) was used to evaluate the actions of the test drugs on motor coordination. The rat was placed on the rotating rod, and the time the rat stayed there was measured. Animals that stayed at least 60 s on the rotating rod before drug administration were accepted to the test, and 60 s was also used as a cutoff time in the test proper.

Possible effects of the drugs on spontaneous locomotor activity were tested in a measurement box (70 × 70 × 35 cm; Kungsbacka Regler & Mätteknik, Kungsbacka, Sweden) isolated from sound and light. Photocells were located at two different levels (2 and 12 cm) above the floor of the box to automatically detect movements of the animal. A 30-min measurement period was started 15 min after the drug injections.

Morphine Tolerance Scheme

Morphine tolerance was induced using a 4-day scheme in which the rats received two daily subcutaneous injections at 10:00 AM and 8:00 PM. The individual doses were 10 mg/kg on day 1, 15 mg/kg on day 2, 20 mg/kg on day 3, and 30 mg/kg on day 4.

Experiment I: Does Ibudilast Increase Acute Morphine-induced Antinociception?

Morphine (2.5 mg/kg) or vehicle combined with three different doses of ibudilast (2.5, 7.5, and 22.5 mg/kg) or vehicle was used to study the effects of ibudilast alone or in acute morphine-induced antinociception.

Experiment II: Could Ibudilast Coadministration Prevent the Development of Morphine Tolerance?

The second experiment was designed to study whether coadministration of ibudilast could attenuate development of morphine tolerance. The effect of repeated ibudilast administrations on the acute antinociceptive effect of morphine was also studied to determine cross-tolerance. On days 1–4, the rats received ibudilast (0.83, 2.5, or 7.5 mg/kg twice daily) or vehicle with or without morphine according to the morphine tolerance scheme. On day 5, antinociception was measured after morphine (5 mg/kg).

After the nociceptive tests on day 5, the effects of ibudilast on morphine withdrawal–induced weight loss were studied. After the acute morphine administration, the treatment was ceased, and the rats were weighed. After 24, 48, and 96 h, the rats were weighed again, and the average weight changes (in grams) were calculated.

The effects of ibudilast on motor coordination were studied in tandem with experiment II, in which the ibudilast pretreatment lasted for 4 days (0.83, 2.5, and 7.5 mg/kg twice daily). The acute effects were assessed on day 1 after the first administration and again on day 4 after 3 days of ibudilast treatment. The rotarod test was performed at 30 and 120 min from drug administration.

Experiment IIIA: Does Acute Ibudilast Administration Restore Morphine-induced Antinociception in Morphine-tolerant Rats?

The effects of acutely administered morphine with or without ibudilast in morphine-tolerant rats were studied. Ibudilast alone and vehicle were administered as controls. Morphine tolerance was induced during days 1–4 using the morphine tolerance scheme. On day 5, antinociception was measured after the rats had received ibudilast (2.5 or 7.5 mg/kg) and/or 5 mg/kg morphine or vehicle. Table 1 clarifies the groups used in this experiment.

Experiment IIIB: Does Chronic Ibudilast Restore Morphine-induced Antinociception in Morphine-tolerant Rats?

We continued from experiment IIIA using the same experimental animals. During days 5–8, the morphine-tolerant rats that were given morphine on days 1–5 received morphine doses of 10 mg/kg twice daily and also ibudilast (2.5 or 7.5 mg/kg twice daily). On day 9, antinociception was measured after the rats had received simultaneous 5 mg/kg morphine and ibudilast (2.5 or 7.5 mg/kg) or vehicle.

We also studied the possible development of tolerance to the antinociceptive effects of ibudilast in morphine-tolerant rats. The rats that had received ibudilast only on day 5 received ibudilast (2.5 or 7.5 mg/kg twice daily) on days 5–8. On day 9, antinociception was measured after the rats had received ibudilast (2.5 or 7.5 mg/kg). Table 1 clarifies the groups used in this experiment.

Statistical Analysis

The hot plate and tail-flick results are expressed as percentage of the maximum possible effect (MPE%), calculated as MPE% = [(postdrug latency − baseline latency)/(cutoff time − baseline latency)] × 100%, which takes into account the differences in baseline nociceptive latencies. In the text and figures, results are presented as means of the groups (±SEM). The data were tested for statistically significant differences in mean values by two-way analysis of variance followed by

Table 1. Experimental Design of Experiments IIIA and IIIB

	Experiment IIIA		Experiment IIIB	
Group	Pretreatment (Days 1–4)	Acute Treatment (Day 5, Morning)	Continuation Treatment (Days 5–8)	Acute Treatment (Day 9, Morning)
1	Vehicle	Vehicle	Vehicle	Vehicle
2	Vehicle	Mo 5 mg/kg	—	—
3	Vehicle	Vehicle	Vehicle	Mo 5 mg/kg
4	Morphine tolerance scheme	Mo 5 mg/kg	Mo 10 mg/kg BID	Mo 5 mg/kg
5	Morphine tolerance scheme	Ib 2.5 mg/kg	Ib 2.5 mg/kg BID	Ib 2.5 mg/kg
6	Morphine tolerance scheme	Ib 7.5 mg/kg	Ib 7.5 mg/kg BID	Ib 7.5 mg/kg
7	Morphine tolerance scheme	Mo 5 mg/kg + Ib 2.5 mg/kg	Mo 10 mg/kg BID + Ib 2.5 mg/kg BID	Mo 5 mg/kg + Ib 2.5 mg/kg
8	Morphine tolerance scheme	Mo 5 mg/kg + Ib 7.5 mg/kg	Mo 10 mg/kg BID + Ib 7.5 mg/kg BID	Mo 5 mg/kg + Ib 7.5 mg/kg

Morphine (Mo) was administered subcutaneously; ibudilast (Ib) was administered intraperitoneally.
BID = twice daily.

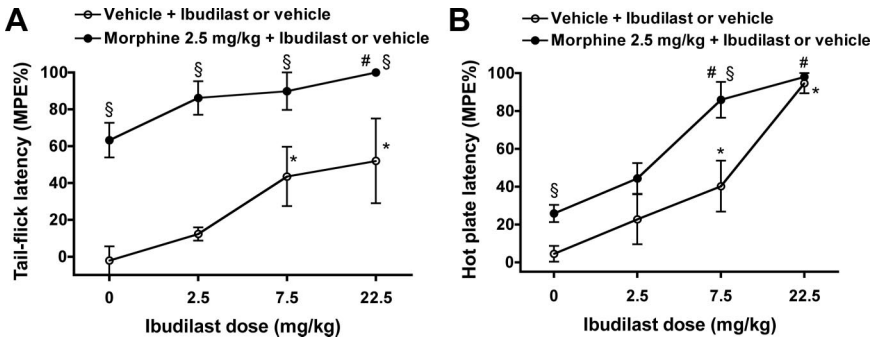

Fig. 1. Experiment I: Effects of ibudilast on acute morphine antinociception in the tail-flick (*A*) and hot plate (*B*) tests. Rats (n = 7 per group) were given the same subcutaneous dose of morphine (2.5 mg/kg) or vehicle with or without three different doses of intraperitoneal ibudilast or vehicle. The mean of the maximum possible effect (MPE%) ± SEM is plotted after 30 min of administration. For the ibudilast groups: * Statistically significant difference ($P < 0.05$) as compared with the group that was given vehicle only. For the morphine groups: # Statistically significant difference ($P < 0.05$) as compared with the group that was given morphine without ibudilast. § Statistically significant difference ($P < 0.05$) as compared with the group that was given the same dose of ibudilast without morphine.

a Bonferroni correction for multiple comparisons (group × dose or group × time). The difference was considered significant at $P < 0.05$ in both the analysis of variance and the *post hoc* test. The data were analyzed using GraphPad Prism, version 4.0c for Macintosh (GraphPad Software, Inc., San Diego, CA).

Results

Experiment I: Does Ibudilast Increase Acute Morphine-induced Antinociception?

In this experiment, antinociception was assessed after administration of three different doses of ibudilast with or without morphine to drug-naive rats.

Vehicle injections had no significant effects on pain thresholds in any of the tests. In the tail-flick test (fig. 1A), ibudilast produced dose-related antinociception (44% MPE with 7.5 mg/kg, 52% MPE with 22.5 mg/kg; $P < 0.05$ compared with vehicle) 30 min after injection. Morphine (2.5 mg/kg) alone produced an antinociceptive effect of 63% MPE ($P < 0.05$) at 30 min. Combined with 22.5 mg/kg ibudilast, antinociception increased to 100% ($P < 0.05$ compared with morphine alone). The combination of morphine with any of the ibudilast doses produced a significantly greater effect compared with when ibudilast was given with the vehicle.

In the hot plate test (fig. 1B), morphine (2.5 mg/kg) alone produced significant antinociception (26% MPE) after 30 min of administration. Ibudilast produced dose-related antinociception from 23% MPE (2.5 mg/kg) to 95% MPE (22.5 mg/kg). The cotreatment of morphine and ibudilast produced a significantly greater MPE% than ibudilast alone at 7.5 mg/kg.

After 120 min of administration, 22.5 mg/kg ibudilast produced significant antinociception alone (49 ± 15% MPE) and combined with morphine (89 ± 10% MPE) compared with vehicle (4 ± 2% MPE) or morphine alone (5 ± 2% MPE) in the hot plate test.

Experiment II: Could Ibudilast Coadministration Prevent the Development of Morphine Tolerance?

During the 4 pretreatment days, the rats received three different doses of ibudilast with or without morphine tolerance treatment. On day 5, antinociception was assessed after morphine administration.

The rats developed clear tolerance to morphine (5 mg/kg) as indicated by a significantly smaller antinociceptive effect (26% MPE) in the tail-flick test (fig. 2A) after repeated morphine administrations compared with an 81% MPE in morphine-naive rats at 30 min after drug

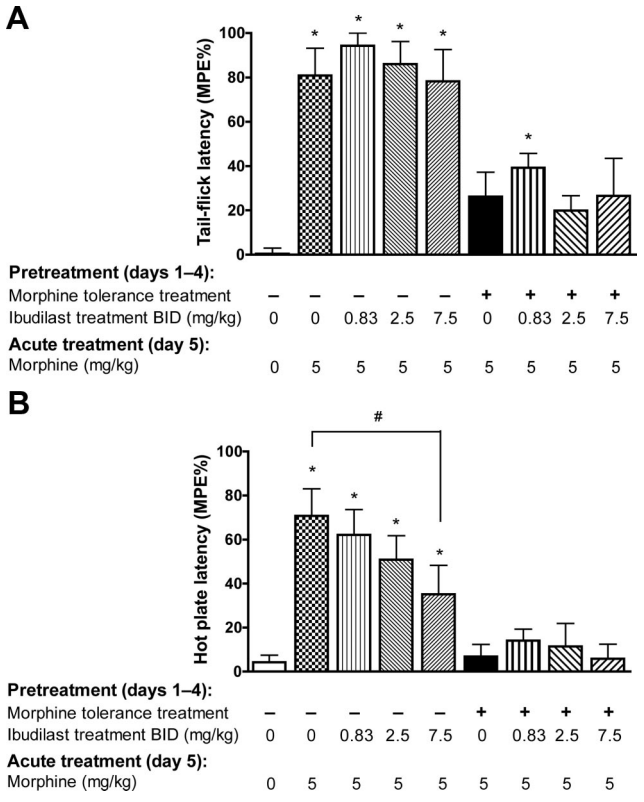

Fig. 2. Experiment II: Effects of ibudilast on development of morphine tolerance. Rats (n = 6 or 7 per group) were treated with morphine tolerance treatment and/or with three different ibudilast pretreatment doses. Acute morphine antinociception was measured using the tail-flick (*A*) and hot plate (*B*) tests. The mean of the maximum possible effect (MPE%) ± SEM is plotted after 30 min of administration. Morphine was administered subcutaneously; ibudilast was administered intraperitoneally. * Statistically significant difference ($P < 0.05$) as compared with the vehicle control. # Statistically significant difference ($P < 0.05$) between selected groups. BID = twice daily.

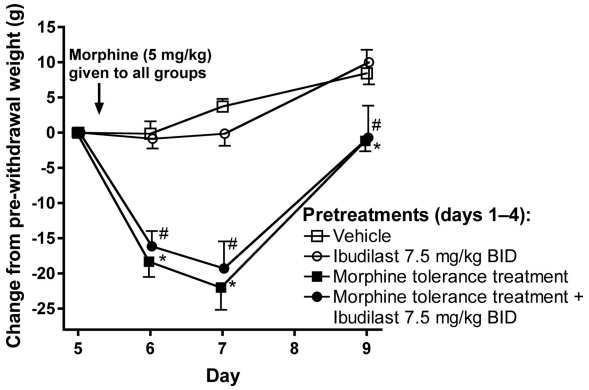

Fig. 3. Weight change during the withdrawal period in ibudilast- and/or morphine-treated rats after experiment II. For 4 preceding days, the rats (n = 6 or 7 per group) were treated with the morphine tolerance scheme and/or with ibudilast (7.5 mg/kg twice daily [BID]). After the acute treatment on day 5, the treatment was stopped and the rats were weighed. The rats were weighed again after 24, 48, and 96 h, and the average weight change in grams ± SEM is plotted in the graph. * Statistically significant difference ($P < 0.05$) against the drug-naive group. # Statistically significant difference ($P < 0.05$) against the morphine-naive, ibudilast-treated group.

administrations. Ibudilast coadministration did not attenuate development of morphine tolerance.

Tolerance developed to the antinociceptive effect of morphine also in the hot plate test (fig. 2B) as morphine (5 mg/kg) produced a 71% MPE in morphine-naive rats and a 7% MPE in morphine-pretreated rats 30 min after drug administration. Coadministration of ibudilast did not prevent the development of morphine tolerance. The antinociceptive effect of morphine was reduced in rats that were pretreated with repeated doses of ibudilast. The reduction in the efficacy of morphine was significant with the highest dose of ibudilast.

During the morphine withdrawal period after experiment II, the average weight gain (fig. 3) was 0.1 ± 1.8 g after 24 h and 3.7 ± 1.1 g after 48 h in the vehicle-pretreated group, whereas the morphine-tolerant group lost a significant mass (−18.3 ± 2.2 g at 24 h and −22.0 ± 3.2 g at 48 h). Ibudilast had no effect on weight loss during withdrawal in the morphine tolerance–treated rats.

Experiment IIIA: Does Acute Ibudilast Administration Restore Morphine-induced Antinociception in Morphine-tolerant Rats?

Rats were treated with the morphine tolerance treatment protocol for 4 days. On day 5, antinociception was measured after administration of morphine and/or two different doses of ibudilast.

In the tail-flick test (fig. 4A), compared with vehicle, 5 mg/kg morphine produced a 90% MPE ($P < 0.05$) in morphine-naive rats and a 23% MPE (nonsignificant) in morphine-tolerant rats 30 min after administration. The administration of ibudilast alone to morphine-tolerant rats did not cause any antinociceptive effect. The coadministration of ibudilast significantly increased the antinociceptive effect of morphine in morphine-tolerant rats (2.5 mg/kg: 59% MPE, 7.5 mg/kg: 100% MPE). At 120 min after administration, the results were in line with the results at 30 min (relevant significant differences are shown in fig. 4B, data not shown for 2.5 mg/kg ibudilast). To allow assessment of the data quality, the mean raw values are presented.

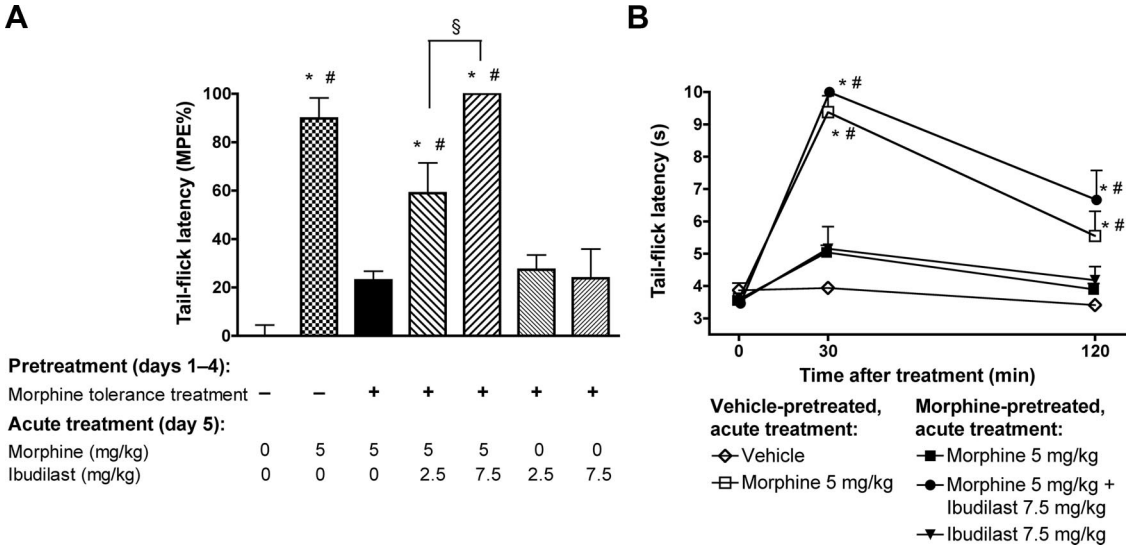

Fig. 4. Experiment IIIA: Effects of acute ibudilast treatment on morphine antinociception in morphine-tolerant rats in the tail-flick test. Morphine-naive rats were given subcutaneous morphine or vehicle, and morphine-tolerant rats were given morphine and/or two different doses of intraperitoneal ibudilast or vehicle. (*A*) The mean of the maximum possible effect (MPE%) ± SEM is plotted for two doses of ibudilast after 30 min of administration. (*B*) Mean tail-flick latencies (in seconds) ± SEM are plotted 30 and 120 min after administration of treatments shown. * Statistically significant difference ($P < 0.05$) as compared with the vehicle control. # Statistically significant difference ($P < 0.05$) as compared with the morphine-tolerant group that was given morphine acutely. § Statistically significant difference ($P < 0.05$) between selected groups. n = 7 per group.

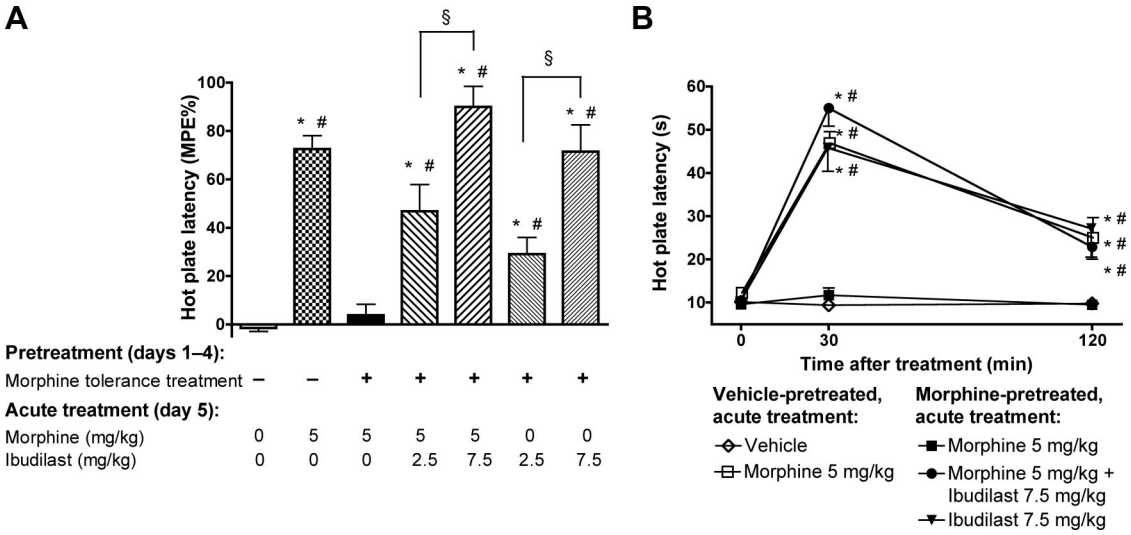

Fig. 5. Experiment IIIA: Effects of acute ibudilast treatment on morphine antinociception in morphine-tolerant rats in the hot plate test. Morphine-naive rats were given subcutaneous morphine or vehicle, and morphine-tolerant rats were given morphine and/or two different doses of intraperitoneal ibudilast or control. (*A*) The mean of the maximum possible effect (MPE%) ± SEM is plotted for two doses of ibudilast after 30 min of administration. (*B*) Mean hot plate latencies (in seconds) ± SEM are plotted for 30 and 120 min after administration of treatments shown. * Statistically significant difference ($P < 0.05$) as compared with the vehicle control. # Statistically significant difference ($P < 0.05$) as compared with the morphine-tolerant group that was given morphine acutely. § Statistically significant differences ($P < 0.05$) between selected groups. n = 7 per group.

In the hot plate test (fig. 5A), 5 mg/kg morphine produced a 73% MPE in morphine-naive rats ($P < 0.05$ compared with vehicle) and a 4% MPE (nonsignificant) in morphine-tolerant rats 30 min after administration. Ibudilast had a dose-related antinociceptive effect in morphine-tolerant rats (2.5 mg/kg: 29% MPE, 7.5 mg/kg: 71% MPE). Coadministration of ibudilast with morphine did not produce a significantly different effect compared with groups that received the same dose of ibudilast only. At 120 min after administration, the results were in line with the results at 30 min also in the hot plate test (relevant significant differences are shown in fig. 5B; data not shown for 2.5 mg/kg ibudilast). To allow assessment of the data quality, the mean raw values are presented.

Experiment IIIB: Is the Effect of Ibudilast Coadministration on Restoration of Morphine-induced Antinociception Described in IIIA Sustained after Repeated Administration?

Rats used in experiment IIIA were further given ibudilast with or without morphine during days 5–8. On day 9, antinociception was assessed using the same acute treatment groups as on day 5.

The results of the tail-flick tests (fig. 6A) were similar to the results in the hot plate test (fig. 6B) at 30 min after drug administrations. Acute morphine produced no antinociception in morphine-tolerant rats and significant antinociception (tail-flick: 77% MPE, hot plate: 58% MPE) in morphine-naive rats compared with vehicle. Acute administration of ibudilast to ibudilast-pretreated rats produced no antinociception compared with vehicle in either of the tests. In the tail-flick test, ibudilast treatment enhanced morphine antinociception in morphine- and ibudilast-cotreated rats, where 2.5 mg/kg ibudilast produced a 21% MPE and 7.5 mg/kg produced a 36% MPE. The respective results with the cotreatment in the hot plate were 14% and 18% MPE, respectively.

Does Ibudilast Affect Motor Coordination or Spontaneous Locomotor Activity?

Effects of ibudilast on motor coordination were controlled during experiment II using the rotarod test. On day 1, all rats (100%) that had received vehicle achieved the cutoff time (60 s) at 30 and 120 min. Intraperitoneal ibudilast in doses of 0.83 and 2.5 mg/kg caused no significant differences (52.3 ± 7.7 and 53.0 ± 7.0 s, respectively) compared with vehicle. After administration of 7.5 mg/kg ibudilast, the mean survival time was 11.2 ± 4.0 s at 30 min and 31.5 ± 12.6 s at 120 min (significant results compared with vehicle). These measurements were repeated on day 4 after three preceding days of ibudilast treatment, and the same significant results were achieved as on day 1.

In a separate experiment, the effect of acutely administered ibudilast and/or morphine on spontaneous motor activity was studied (fig. 7). The rats were placed in a measurement box 15 min after drug administration. At doses of 2.5 and 7.5 mg/kg, compared with vehicle, ibudilast significantly reduced spontaneous locomotor activity (fig. 7) in the measurement box. Combined with 2.5 mg/kg morphine, ibudilast doses of 2.5 and 7.5 mg/kg significantly reduced spontaneous locomotor activity during the whole measurement time compared with morphine alone.

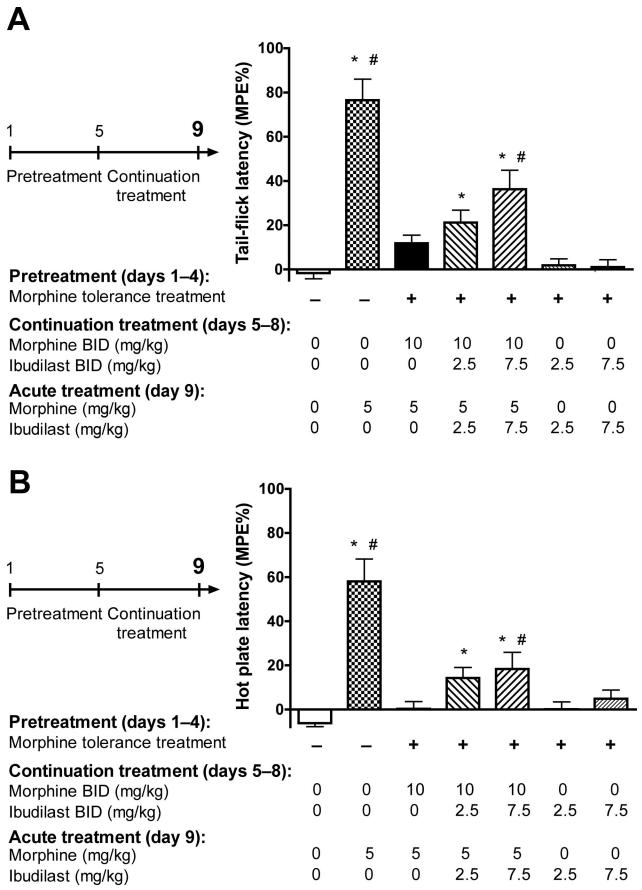

Fig. 6. Experiment IIIB: Effects of chronic ibudilast treatment on morphine antinociception in morphine-tolerant rats. Morphine-tolerant rats were treated with a continuation treatment of morphine and ibudilast, and acute antinociception was measured using the tail-flick (*A*) and hot plate (*B*) tests. This response is compared with the ibudilast-induced antinociception in rats undergoing ibudilast continuation treatment. Morphine was administered subcutaneously; ibudilast was administered intraperitoneally. The mean of the maximum possible effect (MPE%) ± SEM is plotted after 30 min of administration. * Statistically significant difference ($P < 0.05$) as compared with the vehicle control. # Statistically significant difference ($P < 0.05$) as compared with the morphine-treated, morphine-tolerant group (*black bar*). n = 7 per group. BID = twice daily.

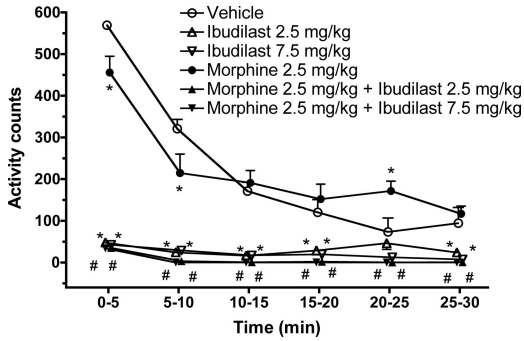

Fig. 7. Effects of ibudilast with or without morphine on spontaneous locomotor activity. The activity counts (number of photocell crossings in 5-min periods) over time ± SEM are shown in 5-min intervals starting 15 min after drug administration. Morphine was administered subcutaneously; ibudilast was administered intraperitoneally. * Statistically significant difference ($P < 0.05$) as compared with the vehicle group. # Statistically significant difference ($P < 0.05$) as compared with the morphine group. n = 6 per group.

Discussion

We focused our research on the effects of ibudilast on opioid-induced antinociception and opioid tolerance. Our objective was to study the effects on ibudilast in several different *in vivo* test setups in both acute and chronic morphine tolerance. These results demonstrate that ibudilast, an inhibitor of glial activation and phosphodiesterase, had antinociceptive effects when administered alone, and it showed increased effects when it was combined with a small dose of morphine in rats. Moreover, ibudilast partly restored the antinociceptive effect of morphine when coadministered to opioid-tolerant animals.

The threshold dose for ibudilast-induced antinociception was 2.5–7.5 mg/kg in the hot plate test, whereas in the tail-flick test its effect was marginal with these doses. Ibudilast also had an additive effect when combined with morphine, particularly in the hot plate test. Supporting our findings, ibudilast has recently been shown to increase the antinociceptive effect of morphine and oxycodone.[22,23] However, ibudilast did not significantly affect hind paw or tail-flick latencies when administered in doses of 7.5 mg/kg intraperitoneally or 50 mg/kg orally alone in those studies. In our study, the 7.5-mg/kg dose of ibudilast caused a marginal (one time significant) increase in the tail-flick but a clear increase in the hot plate latencies after acute administration.

The hot plate test also reflects supraspinal antinociception, whereas the tail-flick test is a spinal reflex.[25,26] The hot plate test may also be more sensitive to unspecific behavioral effects such as sedation. To control that the antinociceptive effects of ibudilast are not due to sedative effects, we studied the effects of ibudilast on motor coordination and locomotor activity. Ibudilast decreased locomotor activity at the doses of 2.5 and 7.5 mg/kg and impaired motor coordination at 7.5 mg/kg. In accord, 7.5 mg/kg ibudilast has been reported to cause transient sedation and decreased reactivity to touch in a previous study.[21] Therefore, we cannot rule out that ibudilast-induced antinociception is, at least partly, caused by reduced locomotor activity and/or impaired motor coordination especially in the hot plate test. The mechanisms by which ibudilast reduces locomotor activity, impairs motor coordination, and causes antinociception are not known. However, it is unlikely that these effects are mediated through actions on microglia, particularly because glial cells are not reactive in normal animals.

The development of opioid tolerance is a major problem in chronic pain management with opioids. We studied the possibility to reverse morphine tolerance by adding ibudilast to morphine once tolerance had already developed. In established morphine tolerance, the combination of ibudilast and morphine induced antinocicep-

tion, which may indicate that ibudilast partly restored the antinociceptive effect of the 5-mg/kg dose of morphine (fig. 4A). Three findings suggest that ibudilast can partly restore opioid antinociception in morphine-tolerant rats in the tail-flick test. First, ibudilast caused a dose-related increase in the tail-flick latency by morphine in opioid-tolerant rats, whereas it did not have any significant independent effect with the studied doses in this experiment (fig. 4A). Second, ibudilast increased the antinociceptive effect of morphine also at the 120-min measurement point in opioid-tolerant rats (fig. 4B). Third, after repeated ibudilast administration, ibudilast did not have any independent effect in the tail-flick test, but cotreatment with morphine partly restored the antinociceptive effect of morphine in tolerant animals (fig. 6A). However, in the hot plate test, ibudilast itself significantly increased the latency, which complicated the evaluation of the interaction with morphine in this test. After repeated ibudilast administration to morphine-tolerant rats, ibudilast had no effect in the hot plate test when administered alone, but the combination of ibudilast and morphine induced significant antinociception. The results suggest that ibudilast coadministration with morphine partly restored the opioid effect in tolerant animals also in the hot plate test (fig. 6B). Taken together, these results support the conclusion that ibudilast may partly restore the efficacy of morphine in opioid-tolerant animals at the spinal cord level.

Glial activation has been proposed to be involved in the development of opioid tolerance and withdrawal symptoms,[9,10,12,16,17] and ibudilast has been reported to inhibit glial activation during morphine treatment.[22] Therefore, we further studied whether coadministration of ibudilast with morphine could affect the development of opioid tolerance. However, ibudilast cotreatment during the morphine pretreatment schedule did not affect the development of morphine tolerance as evaluated with morphine alone. The 4-day ibudilast pretreatment alone attenuated the acute effect of morphine in the hot plate test but not in the tail-flick test, suggesting that ibudilast may induce some tolerance in these conditions in the hot plate test. Therefore, even though ibudilast may partly restore the acute effect of morphine in tolerant animals, it could not attenuate the development of tolerance. Lebeboer *et al.*[23] have reported that animals cotreated with ibudilast and morphine did not lose efficacy of morphine in the chronic constriction injury model of neuropathic pain. They concluded that ibudilast inhibits the development of opioid tolerance. However, because they did not provide data on the effect of morphine alone after cotreatment, these results could also indicate that ibudilast can only restore the effect of morphine during coadministration, in line with what was shown in the current study.

Ibudilast coadministration with morphine did not affect spontaneous withdrawal-induced weight loss. In contrast to our results, ibudilast treatment during morphine pretreatment regimen has been reported to reduce naloxone-precipitated withdrawal symptoms across the 60-min postnaloxone observation period.[22] Moreover, ibudilast treatment suppressed spontaneous withdrawal-induced weight loss when administration was started after development of dependence and continued during the withdrawal period.[22] There were substantial differences in experimental designs in these studies, which might partly explain the different results.

In addition to its own antinociceptive effect, ibudilast also restored the antinociceptive effect of morphine in opioid-tolerant animals as well. These two effects may be mediated through different mechanisms. As discussed previously, the antinociceptive effect of ibudilast may be related to reduced locomotor activity, which cannot be associated to the modulatory effect on glial function. However, to the best of our knowledge, routine screening of ibudilast binding to various target proteins has so far not revealed any receptors and/or target proteins that could mediate the decreased locomotor activity.[21]

The development of morphine dependence and tolerance has been linked to the cyclic acid monophosphate pathway. Activation of μ-opioid receptors leads to inhibition of adenylate cyclase.[27,28] However, repeated administration of morphine may lead to up-regulation of adenylate cyclase, increased cyclic acid monophosphate levels, and superactivation of protein kinase A. This contributes to the development of tolerance[27,28] *via* cyclic acid monophosphate response element–binding protein, a transcription factor[29] that regulates genes responsible for the development of physical dependence.[30] Ibudilast, as an inhibitor of phosphodiesterase, increases cyclic acid monophosphate levels and therefore should acutely attenuate the effects of opioids rather than augment them. Therefore, it seems likely that the demonstrated effect of ibudilast is not linked to phosphodiesterase inhibition in opioid receptor–containing neurons.

Activation of glial cells by opioids may lead to the formation of pronociceptive glial products such as IL-1 and tumor necrosis factor α and attenuation of the antinociceptive effects of morphine.[9,12,17,31,32] Inhibition of release of these glial products should then restore the effect of morphine. Ibudilast has been suggested to modulate glial function. It might suppress activated glia through inhibition of phosphodiesterase enzyme[19] and/or through other mechanisms.[21,23] Ibudilast cotreatment with morphine did not affect the development of morphine tolerance. This result suggests that ibudilast does not affect activation of glia. On the other hand, our results do not rule out the possibility that acute suppression of glia-mediated pronociceptive signaling by ibudilast may explain its ability to restore morphine antinociception when coadministered with morphine to opioid-tolerant animals.

In summary, this study demonstrates that ibudilast may restore the antinociceptive effect of morphine in opioid-tolerant animals after single and repeated administration. However, ibudilast did not prevent the development of opioid tolerance. The exact mechanism of action is not known, but our results do not support the suggestion that ibudilast inhibits the activation of microglia. However, our results do not rule out the possibility that ibudilast might affect the release of pronociceptive mediators and thereby restore the effect of morphine. Ibudilast also reduces locomotor activity, and tolerance may develop to its antinociceptive effects. The mechanisms of these effects should be better understood before ibudilast is considered for clinical use.

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