Selective 5-HT$_{1A}$-R-agonist Repinotan Prevents Remifentanil-induced Ventilatory Depression and Prolongs Antinociception

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ABSTRACT

Background: 5-HT$_{1A}$-R-agonist repinotan was shown to counteract a morphine-induced ventilatory depression but had pronociceptive effects at small doses (0.2 µg/kg). It remained to be clarified (1) whether a moderate dose of repinotan, sufficient to stimulate spontaneous breathing, impairs antinociception if plasma concentration decreases over time, and if (2) moderate doses prevent ventilatory depression if given before the opioid.

Methods: A dose–response curve of the repinotan effects on spontaneous minute ventilation during continuous remifentanil infusion in anesthetized rats was established to identify moderate doses: (1) tail-flick reflex latencies to assess nociception were recorded until 60 min after cessation of a continuous remifentanil infusion with or without a concomitant moderate repinotan dose (10 µg/kg), and (2) remifentanil boluses (2.5 µg/kg) were given after repinotan (10 and 20 µg/kg).

Results: (1) Remifentanil-induced antinociception lasted only 5 min after infusion was stopped (tail-flick reflex latencies; median [interquartile range], 97 [54–100]% of maximum possible effect; P = 0.034), but was extended by repinotan (10 µg/kg) to 30 min (tail-flick reflex latencies, 100 [75–100]% of maximum possible effect; P = 0.031). Repinotan (10 µg/kg) alone did not have any significant antino-

cceptive effect. (2) The ventilatory depression by remifentanil boluses (2.5 µg/kg; minute ventilation, −65 [−81 to −56]%; P = 0.031, n = 5) was blunted by repinotan (20 µg/kg; minute ventilation, −24 [−53 to 13]%; P = 0.313, compared with the pretreatment level).

Conclusions: Repinotan prevented remifentanil-induced ventilatory depression in spontaneously breathing, anesthetized rats. Although repinotan did not depress nociception itself, it prolonged the profound antinociception after discontinuation of remifentanil infusion.

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What We Already Know about This Topic
• Opioid-induced respiratory depression is a dose-limiting side effect in the safe clinical use of opioids
• The serotoninergic receptor type 1A agonist repinotan antagonizes morphine-induced respiratory depression but has a pronociceptive effect at low doses

What This Article Tells Us That Is New
• Repinotan prevented respiratory depression after bolus injection of remifentanil and prolonged, rather than antagonized, antinociception in spontaneously breathing anesthetized rats
• Serotonergic receptor type 1A agonists warrant additional study as opioid adjuncts for their ventilatory effects

VENTILATORY depression is a major devastating adverse event of opioid-based pain therapy and brief opioid-treated painful procedures in the clinic.1,2 The selective, full 5-HT$_{1A}$-R-agonist repinotan3,4 has been shown to counteract morphine-induced ventilatory depression in spontaneously breathing rats.3 Unlike other 5-HT$_{1A}$-R agonists, repinotan is approved for intravenous use in humans and has undergone a series of clinical phase II trials into neuroprotection after traumatic brain injury and stroke.6–9 Nociception, assessed with a tail-flick reflex, was shown to be enhanced with very small doses (0.2 µg/kg), indicating pronociception.5 A moderate dose of repinotan (20 µg/kg), sufficient to stimulate spontaneous breathing, produced a nonsignificant trend toward antinociception; higher doses (200 µg/kg) reached statistical significance. It was not investigated whether moderate repinotan doses have pronociceptive effects with plasma concentrations decreasing over time.

The ultrashort-acting opioid remifentanil is used with bolus injections in a growing number of brief, painful pro-
cerebrospinal fluid (CSF), denervated spinal cord, or dorsal column, with antinociception quickly wearing off after the procedure. Interestingly, the selective 5-HT1A-R-agonist F13640 was shown to reduce postoperative analgesic requirements after remifentanil in rats. The question arises of whether repinotan at moderate doses sufficient to stimulate spontaneous breathing also contributes to opioid analgesia. In addition, it was not investigated if repinotan prevents ventilatory depression if given before opioid administration.

This study was carried out (1) to establish a dose–response curve of repinotan on the counteraction of remifentanil-induced ventilatory depression, (2) to determine the effects of a moderate repinotan dose (10 μg/kg) on nociception after discontinuation of a remifentanil infusion, and (3) to verify if repinotan prevents ventilatory depression if given before remifentanil bolus injections in spontaneously breathing, anesthetized rats.

Materials and Methods

Animals

This study was performed with approval from the local Institutional Animal Review Board for animal research (Cologne, Germany) and in accordance with the "Guide for the Care and Use of Laboratory Animals." The experimental setup has been described in detail elsewhere. In brief, 50 male Sprague-Dawley rats (median weight, 284 g; range, 208–370 g; Charles River GmbH, Sulzfeld, Germany) were deeply anesthetized with a single intraperitoneal injection of sodium-pentobarbitone (60 mg/kg) and placed supine on a heating pad to maintain rectal temperature constantly at 37° ± 0.5°C. The right inguinal artery and vein were cannulated via a small surgical incision for intravenous application of study drugs and continuous monitoring of heart rate (HR) and mean arterial blood pressure (MAP). A tracheostomy was performed, and anesthesia was maintained with sevoflurane as soon as animals produced signs of subsiding barbiturate effect. After intubation of the trachea and establishment of spontaneous breathing, the animals were allowed for acclimation to sevoflurane, except for the group without continuous remifentanil infusion, which had a 60 min period (see fig. 1). The latency of the tail-flick reflex response (tail-flick reflex latency [TFL]), evoked by a 100-W light beam source mounted 15 mm over the base of the tail, was recorded with a strain gauge attached to the tail. The TFL was determined as the distance between the spike artifact evoked by the power-on of the heating source and the tail-flick response detected by the strain gauge. The effect of an antinociceptive drug is the extension of TFL: a shortened TFL indicates enhanced nociceptive responsiveness. According to Nadez and Goodchild, the TFL taken after the drug application is TFLtreatment, and the TFL taken before the drug application is TFLpretreatment. The latency to switch off the heat to avoid damage to the tail skin was set at 15 s in this study (TFLoffset). Thus, the maximum possible effect (MPE) of an antinociceptive drug was the extension of the TFLtreatment to a maximum of 15 s. Results of the TFL are given as percent of MPE (%MPE) and calculated by the formula %MPE = 100 × [TFLtreatment − TFLpretreatment] × [TFLoffset − TFLpretreatment]−1. Enhanced nociceptive responsiveness results in negative %MPE values; antinociception is indicated by positive %MPE values. A complete suppression of the tail-flick reflex, which is no response within 15 s after the onset of heat, is by definition a 100%MPE.

A blood pressure transducer, temperature probe, and strain-gauge transducer were connected to the same analog-to-digital interface as the spirometer.

Drug Application Protocols

A schematic overview of the drug application protocol is given in figure 1.

Dose–response curves on MV of increasing doses of either repinotan (R-(-)-2-[4-[(chroman-2-ylmethyl)-amino]-butyl]-1,1-dioxo-benzo[d]isothiazolone-hydro-chloride; Bayer Healthcare AG, Wuppertal, Germany), or the standard 5-HT1A-R-agonist 8-OH-DPAT ([+]8-Hydroxy-2-(di-N-propylamino)-tetralin; Tocris, Bristol, United Kingdom), or NaCl 0.9% (Braun, Melsungen, Germany) were established during continuous remifentanil infusion (GlaxoSmithKline GmbH & Co. KG, Hamburg, Germany) to delineate moderate doses.

After the identification of 10 μg/kg repinotan as a moderate dose, the effects of such a single dose on TFL after discontinuation of an 80-min infusion of remifentanil, repinotan alone, and remifentanil alone were assessed to 60 min after discontinuation of the continuous remifentanil infusion. The effects of moderate doses of repinotan (10 and 20 μg/kg) on spontaneous breathing given before bolus injections of remifentanil were measured.

Repinotan and Continuous Remifentanil Infusion.

The remifentanil infusion rate was set to achieve a 50% reduction in respiratory frequency. Thereafter, repinotan was injected
Antinociception of Repinotan and Remifentanil

**Table 1.** Remifentanil was infused continuously at a rate targeted to reduce respiratory frequency by more than 50% of the pretreatment level. Remifentanil concentrations are given as median with interquartile range (IQR) (A1–A5). Repinotan (n = 6) or (A2) 8-OH-DPAT (+/-)8-Hydroxy-2-(di-n-propylamino)-tetralin, n = 6) were injected every 15 min at increasing doses during remifentanil infusion; no doses of 8-OH-DPAT higher than 10 µg/kg were attempted because of the fatal cardiocirculatory depression seen in previous experiments (A1). Two series with NaCl (0.9%) to serve as controls were carried out (each n = 6) (A3, A4). After a NaCl (0.9%) series, a single dose of repinotan (10 µg/kg, n = 6) (A3) or no repinotan (n = 6) was added, and tail-flick latencies (TFL) (A4) were taken for 1 h. A single dose of repinotan (10 µg/kg) was given without preceding remifentanil (A5). TFL were taken for 1 h (n = 7). One experiment was prematurely terminated because of technical problems and excluded from analysis. Repinotan was given at doses of 10 µg/kg (n = 6) or 20 µg/kg (n = 5; one experiment was excluded from analysis because of technical problems); NaCl (0.9%) injections served as controls. Remifentanil (bolus, 2.5 µg/kg) was injected 15 min thereafter (B). A

**A remifentanil/ continuous injection**

**B remifentanil/ bolus injection**

Intravenously every 15 min, with doses ranging from 0.1 µg/kg to 100 µg/kg (n = 6, fig. 1, A1). For comparison, the standard 5-HT₁A-R-agonist 8-OH-DPAT was given at doses of 0.1 to 10 µg/kg (n = 6, fig. 1, A2). NaCl 0.9% injections in another series (n = 6, fig. 1, A3) served as controls. TFL were recorded before the start of remifentanil infusion (and taken as pretreatment level), 5 min thereafter, and at the end of the remifentanil infusion. We did not perform TFL measurements between 5-HT₁A-R-agonist administrations because the tail-flick reflex was always abolished with the onset of remifentanil and remained absent throughout preliminary experiments as long as remifentanil was infused continuously. After completion of the remifentanil and NaCl 0.9% series, a single bolus of remifentanil (10 µg/kg, n = 6, fig. 1, A4) was given, and TFLs were determined at 5, 15, 30, 45, and 60 min thereafter. This was done to determine the time profile of the nociceptive effects of remifentanil and remifentanil after 80 min of continuous remifentanil infusion. In an additional series, remifentanil (10 µg/kg) was given without previous remifentanil infusion (n = 7, fig. 1, A5). All experiments were performed by one of the coauthors (D.H.) and a technical assistant. For technical reasons, they were not blinded to treatment groups. Both were unaware of the drug effects and did not participate in the data analysis.

The number of experiments involving increasing doses of remifentanil (n = 6) was based on our previous experience, in which a 20-µg/kg dose of remifentanil counteracted a morphine-induced ventilatory depression (−72 ± 28%; mean ± SE) to 18 ± 58% of the pretreatment level. With an α set at 0.05, the power was calculated as 0.92 with n = 6 experiments in the double-sided power analysis.

**Repinotan and Remifentanil Bolus Injections.** Repinotan (10 µg/kg, n = 6; or 20 µg/kg, n = 5, fig. 1B), or NaCl (0.9%) were administered intravenously. Twenty minutes later, remifentanil was injected intravenously as a bolus of 2.5 µg/kg. This dose was found to be the highest possible to avoid hypoxia in preliminary experiments. Because of the brief measurement intervals upon administration of the remifentanil boluses no TFL were taken in this series of experiments.

**Statistical Analysis**

Pretreatment MV, TFL, MAP, HR, and sevoflurane and remifentanil dosing of matched groups were compared with the Mann–Whitney U test. MV during experiments were

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calculated as change in percent of pretreatment level (% change) according to the formula: \( \% \text{ change} = 100 \times \left( \frac{\text{MV}_{\text{treatment}}}{\text{MV}_{\text{pretreatment}}} - 1 \right) \). This also applies to MAP and HR. TFL were calculated as change in percent of the maximum possible effect (%MPE), as shown in Materials and Methods (see Nociception). MV levels upon repinotan administration were compared with pretreatment levels with Dunn’s multiple comparison test. The same methods were used to compare pretreatment and posttreatment TFL, MAP, and HR. All tests were two-tailed, and \( P < 0.05 \) was considered statistically significant. All data were processed with the Chart 4.0 and Scope 4.0 software package (ADInstruments GmbH). Statistical analyses were performed using Prism 4® software package for Macintosh (GraphPad Software Inc., San Diego, CA) and IBM® SPSS® Statistics, Version 19 (IBM Deutschland GmbH, Ehningen, Germany). The power analysis was done with the Simple Interactive Statistical Analysis (SISA) online software package (Quantitative Skills, Hilversum, The Netherlands).††

**Results**

**Continuous Remifentanil Infusion**

**Spontaneous Breathing.** The median pretreatment MV in the repinotan group was 173 [interquartile range, 149–200] ml/min, 162 [121–171] ml/min in the 8-OH-DPAT group, and 152 [105–201] ml/min in the NaCl (0.9%) group. Sevoflurane concentrations were 3.0 [2.9–3.0] Vol% in the repinotan group, 3.0 [3.0–3.1] Vol% in the 8-OH-DPAT group, and 3.0 [3.0–3.1] Vol% in the NaCl (0.9%) group. Except for a difference in pretreatment MV between the 8-OH-DPAT and the NaCl (0.9%) group (\( P = 0.008 \)), groups did not differ statistically otherwise. Remifentanil depressed MV to \( -56 \% \rightarrow 67 \% \) in the repinotan group (\( P = 0.031 \)). Remifentanil counteracted this ventilatory depression in a dose-dependent manner, as indicated by the MV returning almost to pretreatment level (MV, \(-8 \% \rightarrow 29\% \)) with the moderate dose (10 \( \mu \)g/kg) and to \(-4 \% \rightarrow 35\% \) with the high dose (100 \( \mu \)g/kg). MV remained depressed during controls with NaCl (0.9%) (fig. 2). Given a stable ventilatory depression, the mean ED\(_{50}\) for this repinotan effect was 3.1 [95% CI, 1.0–9.9] \( \mu \)g/kg. We took the threefold ED\(_{50}\) as a moderate dose of remifentanil for additional experiments on nociception. 8-OH-DPAT (10 \( \mu \)g/kg) also counteracted remifentanil-induced ventilatory depression (32 [–6 to 36]%), \( P = 0.156 \), compared with remifentanil concentration). 8-OH-DPAT doses higher than 10 \( \mu \)g/kg were not tolerated hemodynamically in previous investigations and thus were not tested.

**Nociception.** The median pretreatment TFL was 7.5 [6.6–9.1] s; the treatment groups did not differ statistically. During remifentanil infusion, the TFL was always 100%MPE 5 min after onset and throughout remifentanil infusion, meaning that the tail-flick reflex remained completely suppressed (fig. 3).

A single dose of remifentanil (10 \( \mu \)g/kg), injected at the end of continuous remifentanil infusion, prevented the return of the TFL to baseline for at least 30 min (fig. 3). This is indicated by the return of the TFL to baseline 45 min after cessation of remifentanil administration, compared with 5 min in the NaCl (0.9%) group. For comparison, remifentanil (10 \( \mu \)g/kg), administered without a preceding remifentanil infusion, did not affect TFL.

**Remifentanil Bolus Injection**

The median pretreatment MV in the repinotan 10 \( \mu \)g/kg group was 167 [120–207] ml/min, 161 [144–190] ml/min in the repinotan 20 \( \mu \)g/kg group, and 149 [120–190] ml/min in the NaCl 0.9% group. Sevoflurane concentrations were 3.0 Vol% in all groups. There were no statistical significant differences between groups. Figure 4 shows a representative experiment on the prevention of remifentanil-induced ventilatory depression. Figure 5 shows that a remifentanil bolus (2.5 \( \mu \)g/kg) without preceding remifentanil administration depressed spontaneous breathing to \(-64 \% \rightarrow 81\% \) \( P = 0.031 \), compared with the pretreatment level). This ventilatory depression was blunted if remifentanil (10 \( \mu \)g/kg;
Cardiovascular Effects

Remifentanil induced a depression of both MAP and HR, both if administered continuously (table 1) and also as a bolus injection (table 2). Unlike ventilatory effects, MAP reduction was not alleviated by repinotan, if given subsequent to the opioid (table 1) or if given before (table 2). Note that moderate and high doses of repinotan recovered HR because HR changes were not significantly different from the pretreatment level with the 10 and 100 µg/kg dose. Repinotan, given alone, did not show statistically significant effects on MAP and HR (table 2). There were no deleterious cardiovascular complications.

Discussion

This study found that a moderate dose of repinotan (10 µg/kg), given alone, had no intrinsic antinociceptive effect, but it prolonged opioid-induced antinociception after discontinuation of remifentanil to at least 30 min. Repinotan (10 and 20 µg/kg) significantly blunted the ventilatory depression caused by bolus injections of remifentanil. It was also confirmed that the sustained ventilatory depression caused by continuous infusion of remifentanil was antagonized by moderate and higher doses of repinotan (10 and 100 µg/kg). Repinotan had a mild, yet statistically nonsignificant, tendency to induce tachycardia and hypotension and did not produce serious cardiovascular complications even with the highest dose.

The dose–response curve for repinotan counteracting the remifentanil-induced ventilatory depression aimed at verifying whether stimulatory effects in remifentanil-induced ventilatory depression are comparable with those obtained previously with morphine\(^5\) and to identify a moderate dose, sufficient to stimulate spontaneous breathing. An ED\(_{50}\) of 3.1 µg/kg was found in this work, which is in the same range previously found in morphine-induced ventilatory depression.\(^5\) In that work, we reported an ED\(_{50}\) of 1.9 ± 0.5 µg/kg, with the maximum effective repinotan dose being 20 µg/kg. Note that the ED\(_{50}\) given here should be compared with caution because the required condition, a stable ventilatory depression, is difficult to control for, and MV levels upon opioid administration were different (MV remifentanil, −61% vs. morphine −72%).

A moderate dose of 10 µg/kg repinotan was confirmed to counteract ventilatory depression sufficiently and not to stimulate spontaneous breathing if given alone. Spontaneous breathing is controlled by the brainstem respiratory network, among which different types of neuron groups are interconnected via \(\gamma\)-aminobutyric acid and glycinergic receptors, and with excitatory input from the ascending reticular acti-

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**Fig. 3.** Effects of repinotan (REP, 10 µg/kg), injected at the end of 80 min of continuous infusion of remifentanil (REMI, red bars), 80 min continuous remifentanil injection alone (blue bars), and REP alone without preceding REMI (10 µg/kg, green bars) on tail-flick reflex latencies (TFL). TFL (percent of maximum possible effect [%MPE]) are shown 5, 15, 30, 45 and 60 min after stop of REMI. After stop of REMI infusion, the rapid decline of TFL indicates subsiding antinociception if no REP was involved (blue bars). Antinociception was extended to at least 30 min if REP was given at the end of REMI infusion (red bars). Single-dose REP without preceding REMI (green bars) did not induce antinociception. TFL are shown as %MPE (median with interquartile range [IQR]). Two data points in the REP and REMI group at 1:00 h, one in the REP and NaCl (0.9%) group at 0:45 h, and three at 1:00 h were missing because of technical difficulties. **P < 0.01, *P < 0.05, compared with the pretreatment level. Statistics: Friedman’s ANOVA with Dunn’s multiple comparison, compared with pretreatment level.

MV, −47 [−92 to 19]% and repinotan (20 µg/kg; MV, −25 [−53 to 13]% was given before because both MV levels were not statistically different from pretreatment levels (both groups, P = 0.316).

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**Fig. 4.** Animal was breathing spontaneously through a tracheostomy tube. Arrows indicate time injection of remifentanil bolus. Remifentanil (2.5 µg/kg) profoundly depressed spontaneous ventilation, as shown in a slow and irregular respiratory frequency with increased individual tidal volume (\(V_T\) [ml/s]) (A). Repinotan (10 µg/kg) slightly increased \(V_T\) but not respiratory frequency (compared with A). The ventilatory depression upon a bolus of remifentanil (2.5 µg/kg) was markedly blunted (B).
respiratory frequency. This effect is far more pronounced in inhibitory synapses within the respiratory network. The opioid-induced ventilatory depression. (figs. 4 and 5) but strongly activated spontaneous breathing via 5-HT1A-R stimulation, which eventually leads to a shortening of inspiratory burst suppression and thus increased respiratory frequency. This effect is far more pronounced when spontaneous rhythm pattern is disturbed per se. Opioids do not equally depress respiratory neurons; their network effect is among others an inhibition of the off switch of expiratory neurons that activate the inspiratory off turn, was shown to counteract such breathing disturbances synaptically, making it difficult to predict their net effect on nociception. For instance, applied directly to spinal 5-HT1A-R, 8-OH-DPAT decreased vocalization threshold in the paw pressure test in a dose dependent manner but acted as an antinociceptive in the formalin-test. It has been demonstrated in decerebrated rabbits with 5-HT-R-antagonists, that serotonin tonically inhibits spinal reflex pathways by action at spinal 5-HT1A-R. The authors also reported that intrathecal administration of 5-HT1A-R-agonist SWAY100135 was much more effective in enhancing spinal withdrawal reflex than was intravenous administration of the same, suggesting that antinociceptive effects are mediated mostly via central 5-HT1A-R. According to the manufacturer's information, repinotan penetrates the blood–brain barrier after rapid intravenous administration to rats, and the distribution equilibrium between plasma and brain is reached almost immediately after the start of a constant-rate infusion. Given the concentration ratio of brain to plasma of approximately 1.2–2:1 in rats, pronociceptive effects mediated by spinal 5-HT1A-R presumably were overpowered by actions via central 5-HT1A-R, as the latter was suggested to be more effective.

Although there was no detectable antinociceptive effect of repinotan (10 μg/kg), repinotan subsequent to continuous remifentanil infusion delayed the return of the TFI to baseline. This contributes to previous findings, where the highly selective 5-HT1A-R-agonist F13640 alleviated opioid-induced hyperalgesia and neuropathic pain in rats. Intrathecal and intravenous 5-HT1A-R blockade was shown to significantly reduce fentanyl-induced suppression of spinal reflexes. In turn, activation of central 5-HT1A-R agonists might contribute to bulbospinal opioidergic suppression of spinal reflexes, but this remains to be determined.

It was also verified that repinotan at moderate doses (10 and 20 μg/kg) before remifentanil bolus injection prevented a ventilatory depression. Repinotan doses higher than 20 μg/kg were not investigated to minimize baseline ventilatory stimulation before the remifentanil bolus. This enabled us to demonstrate that the prevention of remifentanil-induced depression is a specific effect of repinotan within the neuronal respiratory network rather than a simple elevation of baseline MV.

Repinotan has been investigated intravenously for neuroprotection in humans. Initial favorable neurologic outcomes in patients with stroke or traumatic brain injury were later not confirmed by multicenter studies. Recently, the only commercially available 5-HT1A-R agonist for use in humans, buspirone, failed to counteract opioid-induced ventilatory depression in humans and did not display antinociceptive effects in healthy volunteers. Of note, buspirone is only a partial 5-HT1A-R agonist and available only for oral administration. (i.e., the combination of low-dose enhancement of nociception followed by high-dose inhibition of nociception) remains in part unknown. 5-HT1A-R-agonists are located in the brain and spinal cord presynaptically and postsynaptically, making it difficult to predict their net effect on nociception. For instance, applied directly to spinal 5-HT1A-R, 8-OH-DPAT decreased vocalization threshold in the paw pressure test in a dose dependent manner but acted as an antinociceptive in the formalin-test. It has been demonstrated in decerebrated rabbits with 5-HT-R-antagonists, that serotonin tonically inhibits spinal reflex pathways by action at spinal 5-HT1A-R. The authors also reported that intrathecal administration of 5-HT1A-R-agonist SWAY100135 was much more effective in enhancing spinal withdrawal reflex than was intravenous administration of the same, suggesting that antinociceptive effects are mediated mostly via central 5-HT1A-R. According to the manufacturer's information, repinotan penetrates the blood–brain barrier after rapid intravenous administration to rats, and the distribution equilibrium between plasma and brain is reached almost immediately after the start of a constant-rate infusion. Given the concentration ratio of brain to plasma of approximately 1.2–2:1 in rats, pronociceptive effects mediated by spinal 5-HT1A-R presumably were overpowered by actions via central 5-HT1A-R, as the latter was suggested to be more effective.

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Table 1. Effects of Repinotan and 8-OH-DPAT during Continuous Remifentanil on MAP and HR

<table>
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<th>MAP (% change)</th>
<th>HR (% change)</th>
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<tr>
<td></td>
<td>Median [IQR]</td>
<td>P Value</td>
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<tr>
<td>Remifentanil (continuous)</td>
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<tr>
<td>REP 0.1 µg/kg</td>
<td>−26 [−33 to −21]</td>
<td>&lt;0.0001</td>
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<td>REP 1 µg/kg</td>
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<td>8-OH 1 µg/kg</td>
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<td>8-OH 10 µg/kg</td>
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<td>0.013</td>
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<tr>
<td>NaCl (0.9%) Second application</td>
<td>−16 [−26 to −7]</td>
<td>0.013</td>
</tr>
<tr>
<td>NaCl (0.9%) Third application</td>
<td>−12 [−23 to −6]</td>
<td>0.013</td>
</tr>
<tr>
<td>NaCl (0.9%) Fourth application</td>
<td>−18 [−21 to −5]</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Effects of repinotan (REP), 8-OH-DPAT (8-OH), and NaCl (0.9%) in combination with continuous infusion of remifentanil on mean arterial pressure (MAP) and heart rate (HR). During remifentanil infusion, concomitant application of REP and 8-OH at small doses (0.1 and 1 µg/kg) did not further change MAP or HR. Moderate (10 µg/kg) and high doses (100 µg/kg) of REP returned HR, but not MAP, almost to the pretreatment level. 8-OH-DPAT (100 µg/kg) was not investigated because serious cardiovascular side effects were seen previously. The NaCl 0.9% group indicates sustained depression of MAP and HR throughout continuous remifentanil infusion. MAP and HR are given as median % change of the pretreatment level (interquartile range [IQR]). Statistics, Friedman’s repeated measures analysis of variance with Dunn’s multiple comparison test, compared with pretreatment level.

* HR in two experiments of the 8-OH-DPAT group was unable to be analyzed because of technical difficulties. All other groups had N = 6.

take. Our findings suggest that intravenous 5-HT1A-R agonists should be studied for nociceptive and ventilatory effects.

The cardiovascular effects upon repinotan administration alone were nonsignificant statistically. In combination with remifentanil, which alone depressed MAP and HR, repinotan did not further aggravate hypotension, except for the highest dose (100 µg/kg). This effect should be interpreted with caution because the fourth NaCl (0.9%) injection also was associated with an MAP reduction (table 1). In addition, 8-OH-DPAT was associated with hypotension with moderate doses (1 and 10 µg/kg). Others saw that 5-HT1A-R-agonist 8-OH-DPAT even prevented arterial hypotension induced by remifentanil in conscious, nonanesthetized rats. It has also been reported that the 5-HT1A-R-agonist 8-OH-DPAT even prevented arterial hypotension.

In this study, the concentration of the anesthetic had to be adjusted to achieve a similar antinociception of Remifentanil and Repinotan. Antinociception of Repinotan and Remifentanil during Continuous Remifentanil on MAP and HR

Table 2. Effects of Remifentanil Bolus Injections after Repinotan Administration on MAP and HR

<table>
<thead>
<tr>
<th></th>
<th>MAP (% change)</th>
<th>HR (% change)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median [IQR]</td>
<td>P Value</td>
</tr>
<tr>
<td>REP 10 µg/kg (n = 6)</td>
<td>−11 [−18 to −6]</td>
<td>0.035</td>
</tr>
<tr>
<td>REMI 2.5 µg/kg (bolus)</td>
<td>−27 [−50 to −11]</td>
<td>0.269</td>
</tr>
<tr>
<td>Control (20 min later)</td>
<td>1 [−11 to 3]</td>
<td>0.674</td>
</tr>
<tr>
<td>REP 20 µg/kg (n = 5)</td>
<td>−14 [−29 to −9]</td>
<td>0.053</td>
</tr>
<tr>
<td>REMI 2.5 µg/kg (bolus)</td>
<td>−28 [−34 to −9]</td>
<td>0.099</td>
</tr>
<tr>
<td>Control (20 min later)</td>
<td>7 [−1 to 9]</td>
<td>0.144</td>
</tr>
<tr>
<td>NaCl (0.9%) (n = 6)</td>
<td>−2 [−11 to 3]</td>
<td>0.393</td>
</tr>
<tr>
<td>REMI 2.5 µg/kg (bolus)</td>
<td>−26 [−30 to −12]</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Control (20 min later)</td>
<td>3 [−10 to 8]</td>
<td>0.393</td>
</tr>
</tbody>
</table>

Effects of repinotan (REP) and bolus repinotan (REMI) on mean arterial pressure (MAP) and heart rate (HR). REP (10 and 20 µg/kg) alone slightly depressed MAP but not HR. Subsequent REMI boluses (2.5 µg/kg) depressed HR, but additional MAP changes did not reach statistical significance. If no REP was given before, REMI depressed MAP and HR. The “control” rows give MAP and HR changes 15 min after bolus injections of REMI or NaCl (0.9%). Both MAP and HR returned to pretreatment levels. MAP and HR are given as median % change of pretreatment level (interquartile range [IQR]). Statistics, Friedman’s repeated measures analysis of variance with Dunn’s multiple comparison test, compared with pretreatment level. P < 0.05 was considered statistically significant.
Some limitations of this study warrant comment. First, an acute, specific tolerance to remifentanil has been suggested by several clinical investigators, whereas others have not found such effect. In our study, the remifentanil concentrations and the anesthetic sevoflurane remained constant once they were leveled to achieve a sustained respiratory depression. Remifentanil dosage was targeted to produce a sustaining ventilatory depression, which led to a complete suppression of the tail-flick reflex in every experiment. Thus, a gradual attenuation of remifentanil-induced antinociception by development of an acute opioid tolerance was hardly detectable by the methods used here.

Second, we did not aim at studying doses of repinotan smaller than the one used here because the scope of this study was the effects of the smallest possible dose to stimulate spontaneous breathing. Again, because 5-HT1A-R is variously located within the trajectories of nociceptive processing, they were reported to enhance or suppress polysynaptic nociceptive reflexes, depending on the experimental setup.

Thus, with nociceptive models other than the one used here, there may be activation by small doses of 5-HT1A-R agonists, which may not be overpowered by opioids. This should be by further studied.

Third, we investigated spontaneously breathing rats anesthetized with pentobarbital and sevoflurane. These substances have marked effects on γ-aminobutyric acid receptors, which are also involved in the respiratory network. It is conceivable that the effects of 5-HT1A-R-agonists may be different in nonanesthetized mammals. However, we have reported on the effects of the 5-HT1A-R-agonist 8-OH-DPAT in a perfused, nonanesthetized, brainstem spinal cord preparation and concluded that the stimulatory effect of 5-HT1A-R-agonists is independent from interaction with γ-aminobutyric acid receptors.

Fourth, the experimental setup did not aim at investigating specific serotonergic side effects, such as the serotonergic syndrome. It is characterized by symptoms such as headache, nausea and vomiting, flush, tachycardia, and agitation in humans and may even aggravate in coadministration with morphine. These conditions have to be reconsidered when planning additional clinical studies involving 5-HT1A-R agonists and may limit the generalizability of the reported effects. However, in the current study, hypertension or tachycardia were not seen.

Conclusions
The 5-HT1A-R-agonist repinotan prevented remifentanil-induced ventilatory depression in spontaneously breathing, anesthetized rats. Although a single dose of repinotan alone (10 µg/kg) did not show intrinsic antinociception, it prolonged the opioid-induced antinociception after discontinuation of remifentanil infusion. 5-HT1A-R-agonists should be the subject to additional research regarding their ventilatory and antinociceptive effects.

References

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