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Effects of Acute and Repeated Treatment with the Biased Mu Opioid Receptor Agonist TRV130 (Oliceridine) on Measures of Antinociception, Gastrointestinal Function & Abuse Liability in Rodents

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Abstract

Rationale—TRV130 (oliceridine; *N*-[(3-methoxythiophen-2-yl)methyl]-2-[(9*R*)-9-pyridin-2-yl-6-oxaspiro[4.5]decan-9-yl]ethanamine) is a novel mu opioid receptor (MOR) agonist that preferentially activates G-protein vs. β-arrestin signaling pathways coupled to MORs. Prevailing evidence suggests that TRV130 and other G-protein-biased MOR agonists may produce therapeutic analgesic effects with reduced adverse effects compared to existing MOR agonists.

Objectives—This study compared effects of acute and repeated TRV130 administration on measures of antinociception, gastrointestinal function, and abuse liability in rodents. We hypothesized that TRV130 would produce robust and sustained antinociception and abuse-related effects during repeated treatment, but that tolerance would develop to GI inhibition.

Methods—Antinociception was assessed using a warm-water tail-withdrawal procedure in mice. Gastrointestinal function was assessed in mice using an in vivo measure of fecal output and in vitro assays of colonic propulsion and of colon and ileum circular muscle contraction. Abuse liability was assessed in rats using an intracranial self-stimulation (ICSS) procedure. (+)-TRV130 was administered with acute and repeated dosing regimens, and (−)-TRV130 was also examined in the ICSS procedure to assess stereoselectivity.

Results—Acute (+)-TRV130 treatment produced robust antinociception, complete inhibition of gastrointestinal function, and weak abuse-related effects. Repeated (+)-TRV130 treatment failed to produce tolerance to antinociception or GI inhibition, and abuse-related effects were enhanced by repeated treatment. Effects of acute and repeated (+)-TRV130 in these procedures resemble effects

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of morphine, with the exception that TRV130 antinociception was more resistant to tolerance. (−)-TRV130 was inactive.

Conclusions—These results suggest that TRV130 retains undesirable constipating and abuse-related effects during repeated treatment despite its bias for G-protein signaling.

Keywords

TRV130; analgesia; constipation; gastrointestinal inhibition; intracranial self-stimulation; abuse liability

INTRODUCTION

Mu opioid receptor (MOR) agonists are effective analgesics across a broad range of conditions, but their use is limited by side effects that include abuse liability and inhibition of gastrointestinal motility. A growing body of evidence suggests that different MOR agonist effects may be mediated by different second-messenger pathways coupled to MOR receptors (Raehal and Bohn 2014; Violin et al. 2014). TRV130 (oliceridine; *N*-[(3-methoxythiophen-2-yl)methyl]-2-[(9*R*)-9-pyridin-2-yl-6-oxaspiro[4.5]decan-9-yl]ethanamine) is a novel mu opioid receptor agonist that selectively activates MOR signaling via G-proteins as opposed to β-arrestins, and this bias toward G-protein signaling has been linked to bias in production of MOR-mediated effects on physiology and behavior (Chen et al. 2013; DeWire et al. 2013; Soergel et al. 2014a; Soergel et al. 2014b; Viscusi et al. 2016). For example, TRV130 was reported to produce robust antinociception with reduced signs of constipation and respiratory depression in rats and mice (DeWire et al. 2013), raising the possibility that TRV130 or other G-protein-biased MOR agonists might function as effective analgesics with fewer side effects than existing nonbiased MOR agonists like morphine. With regard to abuse liability, a recent study found that a single dose of TRV130 failed to produce conditioned place preference in mice (Manglick et al., 2016). However, TRV130 increased morphine-like subjective effects in humans (Soergel et al. 2014a), and β-arrestin 2 knockout enhanced the rewarding effects of morphine in a place-conditioning procedure in mice (Bohn et al. 2003). These findings suggest that bias toward G-protein signaling may not eliminate abuse-related effects of MOR agonists.

In addition to its potential contribution to acute MOR agonist effects, β-arrestin recruitment is an early step in MOR desensitization and internalization, and these processes ultimately contribute to adaptions in receptor function and drug effects during chronic treatment (DeWire et al. 2007; Raehal and Bohn 2014). For example, morphine antinociceptive tolerance was attenuated by genetic deletion of β-arrestin 2 in mice (Raehal and Bohn 2011), and antinociceptive effects of the G-protein-biased MOR agonist herkinorin were resistant to tolerance during chronic treatment (Lamb et al. 2012). These findings suggest that β-arrestin 2 recruitment contributes to MOR agonist antinociceptive tolerance. Conversely, β-arrestin recruitment appears to play a different role in the constipating effects of chronic MOR agonist treatment. In contrast to antinociception, MOR agonist-induced constipation is relatively resistant to tolerance, and this resistance is especially evident to MOR agonist effects on colon vs. ileum function (Akbarali et al. 2014). Moreover, β-arrestin 2 knockout enabled tolerance to morphine effects on colon function (Kang et al. 2012; Maguma et al.

2012). These findings suggest that G-protein bias may protect against tolerance to antinociceptive effects while also facilitating tolerance to undesirable constipating effects of MOR agonists, but this hypothesis has not been examined with chronic administration of G-protein-biased agonists like TRV130.

Repeated treatment also modulates expression of abuse-related effects of MOR agonists. For example, intracranial self-stimulation (ICSS) is one experimental procedure that has been used for preclinical abuse liability assessment (Negus and Miller 2014). ICSS is an operant procedure in which subjects lever press for pulses of electrical brain stimulation delivered via a chronically implanted microelectrode into a brain reward area, and most drugs of abuse increase (or "facilitate") low rates of ICSS maintained by low magnitudes of brain stimulation. At doses up to those that recruit behavioral depression, morphine and other MOR agonists produce only weak ICSS facilitation in opioid-naïve rats; however, repeated MOR agonist administration produces tolerance to behavioral depressant effects and an increase in ICSS facilitation, suggestive of enhanced abuse liability with repeated dosing (Altarifi and Negus 2011; Altarifi et al. 2013). The role of G-protein and β-arrestin signaling in MOR agonist-induced ICSS facilitation and depression has not been investigated, and the implications of biased signaling for effects of repeated dosing on abuse liability are unknown.

To address these issues, the present study examined effects of acute and repeated TRV130 on measures of antinociception, gastrointestinal function, and abuse liability in mice and rats. We hypothesized that repeated TRV130 treatment would produce sustained antinociception but tolerance to any initial signs of inhibited gastrointestinal transit. Effects of acute and repeated TRV130 administration on ICSS were more difficult to predict; however, given evidence that β-arrestin knockout enhances morphine-induced conditioned place preference in mice (Bohn et al. 2003), we hypothesized that acute TRV130 would produce facilitate ICSS, and that this effect would be sustained during repeated treatment.

METHODS

Subjects

Gastrointestinal function was studied in adult male Swiss-Webster mice (Harlan, Indianapolis, IN) housed in groups of up to 3 mice per cage. Intracranial self-stimulation studies used individually housed adult male Sprague-Dawley rats (Harlan, Frederick, MD). All animals were maintained on a 12-hour light/dark cycle (lights on 6:00 a.m. to 6:00 p.m.). Food and water were continuously available except during experimental sessions. Animal maintenance and research were in compliance with National Institutes of Health guidelines on care and use of animal subjects in research, and all animal use protocols were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee.

Studies of Antinociception and Gastrointestinal Function

In vivo studies—Mice were habituated to the housing facility for one week, and prior to any treatments, baseline warm-water tail-withdrawal latencies were determined in each mouse as described below. Mice were then divided into three groups that received repeated

treatment with vehicle (N=10), 10 mg/kg (+)-TRV130 (N=5), or 50 mg/kg morphine (N=5) via subcutaneous (SC) injection three times per day (7 am, 1 pm and 7pm) for three consecutive days. Testing was conducted on Day 4 between 9am and noon. To test for the development of antinociceptive tolerance, saline-treated mice were further subdivided into two groups that received an additional challenge injection on Day 4 of either vehicle (N=5) or 10 mg/kg (+)-TRV130 (N=5), whereas chronic (+)-TRV130-treated mice received an additional challenge injection of 10 mg/kg (+)-TRV130, Chronic morphine-treated mice received an additional challenge injection of 10 mg/kg morphine. This type of treatment regimen has been used previously to assess antinociceptive tolerance in mice (e.g. (Vonvoigtlander et al. 1983), and doses of (+)-TRV130 and morphine selected as repeated and challenge doses were based on prior publications describing potency and effectiveness of these drugs on thermal nociception and constipation in mice (DeWire et al. 2013; Hull et al. 2013; Ross et al. 2008).

Tail-withdrawal latencies were redetermined 20 min after Day 4 challenge injections. For each mouse, the distal 1/3 of the tail was immersed in 52°C ± 0.1°C water, and the latency to tail withdrawal was recorded up to a maximum of 10 s (10 s cutoff). All mice had baseline latencies of 2–4 s (2.57 ± 0.17 sec for all mice in the study). Antinociception was quantified as the percentage of the maximal possible effect (%MPE) according to the equation %MPE = [(test latency-baseline latency)/(10-baseline latency)]×100. Ten min after determination of warm-water tail-withdrawal latencies on Day 4, mice were placed in individual cages with wire mesh floors for 60 min to assess fecal output. The total weight of fecal output was expressed as a fraction of the amount of food consumed over the previous 24 h according to the equation Total Corrected Fecal Output = fecal output in mg / food consumed in previous 24 h in mg. Because mice were housed in groups, the amount of food consumed by all mice in a given cage was divided by the number of mice in that cage to yield an estimated measure of food consumed by each mouse. All mice in a given cage received the same treatment. Mean food consumption levels per mouse over the 24h before testing in each group were: VEH + VEH: 3.6 g/mouse; VEH +TRV130: 3.8 g/mouse; TRV130 + TRV130: 2.8g/mouse; MOR + MOR: 2.4g/mouse.

In vitro studies: colonic propulsion—The acute effects of (+)-TRV130 on colonic propulsion were determined in vitro by video imaging using the gastrointestinal motility monitor system (Catamount Research and Development Inc., Vermont, USA). The entire colon(2 cm from the anus to the ileocaecal valve) was excised, flushed, and placed in an illuminated organ bath continuously perfused with Krebs solution (containing in mM: 118 NaCl, 4.6 KCl, 1.3 NaH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 11 glucose, and 2.5 $CaCl_2$) at 35.5 ± 0.5°C and bubbled with carbogen gas (95% O_2/5% CO_2). The colon was anchored with pins at the mesenteries of the anal and oral ends to prevent interference with pellet movement and over-manipulation of the tissue. The remaining mesenteries were gently trimmed. Colon function was assessed by inserting an artificial clay pellet at the oral end and monitoring the pellet propulsion distance down the length of the colon. A baseline assessment was conducted with each colon prior to treatment initiation. Subsequently, treatment was initiated for the remainder of the study with Krebs solution, (+)-TRV130 (30–100 nM) or morphine (500 nM – 1 μM) (N=5–10 per treatment). Fifteen min after treatment

onset, colon function was redetermined, and the distance travelled by the pellet was video recorded for a maximum of 1 h. The maximum distance of pellet propulsion was determined from the spatiotemporal maps and expressed as a percentage of the baseline propulsion distance in the absence of drug.

In vitro studies: colonic and ileal circular muscle contraction—Isometric tension recordings were carried out on circular muscles of the colon and ileum, and contractions elicited by repeated exposure to 100 nM (+)-TRV130 were determined using methods reported previously to examine effects of repeated morphine (Kang et al. 2012; Ross et al. 2008). Briefly, mice were sacrificed by cervical dislocation, and whole colon and ileum were removed, flushed of their luminal contents, and placed in Krebs solution maintained at 37°C and bubbled continuously with carbogen gas. One-half cm circular rings of tissue were dissected, flushed of their contents, trimmed of mesentery, and mounted in 15 ml or 25 ml heated, siliconized organ baths. Tissues were suspended in the axis of the circular muscle with a metal triangle connected to a force transducer (GR-FT03: Radnoti, Monrovia, CA). Isometric tension was recorded onto a computer using AcqKnowledge 3.82 software (BIOPAC Systems, Inc., Santa Barbara, CA). After tissue equilibration for 60 min, the effects of (+)-TRV130 were examined during four repeated exposures. For each exposure, tissues were incubated in 100 nM (+)-TRV130 for 3 min followed by a 30-min wash out. During wash-out periods, tissues were rinsed in Krebs solution every 6 min. The integral of the first TRV130-induced contraction was set as 100%, and subsequent responses were expressed as a percentage of the initial response.

Data Analysis—For all in vivo and in vitro studies, treatment effects were evaluated by a one-way ANOVA. A significant ANOVA was followed by Bonferroni's post-hoc test as indicated. Significance was defined as $P<0.05$.

Studies of Intracranial Self-Stimulation (ICSS)

Behavioral Procedure and Training—Training and testing procedures were identical to those described previously for other opioid agonists (Altarifi and Negus 2011; Altarifi et al. 2013; Altarifi et al. 2015; Miller et al. 2015). Briefly, experiments were conducted in operant conditioning chambers (Model ENV-007-VP-CT, Med Associates) equipped with a single response lever, stimulus lights, house light, and an ICSS stimulator (Model SG-510, Med Associates) that delivered brain stimulation via an electrode implanted in the left medial forebrain bundle at the level of the lateral hypothalamus (2.8 mm posterior and 1.7 mm lateral from the bregma, and 8.8 mm below the skull). The house light was illuminated during behavioral sessions, and responding under a FR 1 schedule produced a 0.5 s train of 0.1 ms square-wave cathodal pulses together with 0.5 s illumination of stimulus lights over the response lever. The terminal schedule consisted of sequential 10 min components. During each component, one of a descending series of 10 current frequencies was presented (158-56 Hz in 0.05-log increments), with a 60 s trial at each frequency. Each frequency trial consisted of a 10 s timeout, during which five noncontingent stimulations were delivered at the frequency available during that trial, followed by a 50 s "response" period, during which responding produced electrical stimulation. Training was considered to be complete when two criteria were met. First, rats had to respond at rates greater than 50% MCR (see Data

Analysis) during the first three frequency trials of all components for three consecutive days. Second, rats were then tested with saline until these injections had no effect on frequency-rate curves as determined by two-way analysis of variance (see Data Analysis).

Testing—Testing was conducted in two phases. The first phase compared acute effects produced by the (−) and (+) isomers of TRV130 in one group of 6 rats. For these studies, test sessions consisted of three sequential "baseline" components followed first by a time-out period when drug was administered as described below and then by two sequential "test" components. Test sessions were completed on Tuesdays and Fridays, and three-component training sessions were conducted on all other weekdays. The sequence of testing was as follows: (1) (−)-TRV130, with order of doses (vehicle, 1–10 mg/kg) randomized across rats using a Latin-square design, (2) 10 mg/kg cocaine as a positive control, (3) (+)-TRV130, with order of doses (vehicle, 0.1–1.0 mg/kg) randomized across rats using a Latin-square design, and (4) 0.1 mg/kg naltrexone + 1.0 mg/kg (+)-TRV130 to assess sensitivity of (+)-TRV130 effects to opioid receptor antagonism. For studies 1–3, the time-out period during test sessions lasted 30 min, and drugs were administered subcutaneously (SC) either 30 min (TRV130) or 10 min (cocaine) before initiation of test components. For study 4, the time-out period lasted 40 min, and naltrexone was administered intraperitoneally (IP) 10 min before SC administration of (+)-TRV130, which was administered 30 min before initiation of test components.

The second phase of testing evaluated effects of (+)-TRV130 before and after repeated daily dosing with either vehicle, 1.0 mg/kg/day (+)-TRV130, or 3.2 mg/kg/day morphine in three separate groups of 5–6 rats/group. Rats were tested over a period of nine days. On Days 1 and 9, rats received vehicle and cumulative doses of (+)-TRV130. Cumulative dosing sessions consisted of three baseline components followed by 4 cycles of drug administration and testing. Each cycle lasted 50 min and consisted of a 30-min time-out period followed by two 10-min ICSS test components. Vehicle was administered at the beginning of the time-out period for the first cycle, and increasing doses of (+)-TRV130 were administered at the beginning of the next three cycles such that each dose increased the total cumulative dose by 0.5 log units (0.32, 1.0 and 3.2 mg/kg). On intervening days 2–8, rats received daily injections of vehicle, 1.0 mg/kg (+)-TRV130, or 3.2 mg/kg morphine. These treatment sessions consisted of three baseline components followed first by a 30-min time-out period and then by two ICSS test components. Each daily drug dose was administered at the start of the time out period. The morphine dose selected for repeated dosing (3.2 mg/kg/day) was based on previous studies that showed effectiveness of this dose to produce tolerance to morphine-induced rate-decreasing effects and increased expression of abuse-related rate-increasing effects (Altarifi and Negus 2011; Altarifi et al. 2013; Altarifi et al. 2015; Miller et al. 2015). 1.0 mg/kg (+)-TRV130 was selected for repeated dosing because it produced acute effects similar to those of 3.2 mg/kg morphine.

Data Analysis—The primary dependent variable was the reinforcement rate in stimulations/trial during each frequency trial. To normalize these raw data, reinforcement rates from each trial were converted into the percent maximum control rate (% MCR). For initial dose-effect testing, the MCR was determined during the baseline components of each

daily test session and was defined as the mean of the maximal rates observed in any frequency trial during the second and third baseline components. Thus, % MCR for each trial was calculated as (reinforcement rate during a frequency trial / MCR) × 100. Normalized data from the frequency trials of consecutive test components were then averaged across rats for display and for statistical analysis using two-way repeated measures analysis of variance (ANOVA), with drug dose and ICSS frequency as the two factors. A significant ANOVA was followed by a Holm–Sidak post-hoc test. To provide an additional summary of ICSS performance, the total number of stimulations delivered across all 10 frequency trials was determined for each component. The average number of total stimulations per test component was expressed as a percentage of the average number of total stimulations per component during the second and third baseline components (% baseline), and these data were analyzed by one-way ANOVA followed by Bonferroni post hoc test or by t-test as appropriate.

Data for repeated drug treatment were analyzed using a similar approach, with the exception that baseline data for maximum control rate (MCR) and total number of stimulations per component were culled from the second and third components of the three baseline sessions before any drug treatment (six total predrug baseline components). Frequency-rate curves for each cumulative dose-effect curve were examined by two-way repeated measures ANOVA with dose and frequency as the two factors. In addition, the average number of total stimulations per test component was determined for each cumulative (+)-TRV130 dose before and after repeated treatment with saline, (+)-TRV130 or morphine and expressed as a percentage of the pre-drug baseline. These data were analyzed by two-way ANOVA with (+)TRV130 dose and day of testing (Day 1 or Day 9) as the two factors. For both types of analysis, a significant ANOVA was followed by a Holm–Sidak post-hoc test. For all statistical analyses, the criterion for significance was set at $P < 0.05$, and drug induced increases in ICSS (i.e. facilitation of ICSS) was interpreted as an abuse-related effect (Negus and Miller 2014).

Drugs

TRV130 isomers were synthesized by B.E. Blough and dissolved in a vehicle of 1:1:8 ethanol:cremaphor:sterile water. Morphine sulfate, naltrexone HCl, and cocaine HCl (National Institute on Drug Abuse Drug Supply Program, Bethesda, MD) were dissolved in saline.

RESULTS

Antinociception and Gastrointestinal Function

Figure 1 shows the antinociceptive effects (Fig. 1A) and constipating effects (Fig. 1B) of (+)-TRV130 in mice after acute and repeated treatment. Statistical results for this and all other figures are reported in the figure legends. In the repeated-vehicle treatment group, vehicle administration on Day 4 did not produce antinociception, and mice displayed high fecal output, whereas 10 mg/kg (+)-TRV130 produced maximal antinociception in all mice and nearly maximal suppression of fecal output. In the 3-day (+)-TRV130 treatment group, a challenge dose of 10 mg/kg (+)-TRV130 continued to produce maximal antinociception and

near-maximal suppression of fecal output, indicative of a lack of tolerance development. Conversely, in the 3-day morphine treatment group, tolerance developed to morphine antinociception but not to morphine-induced suppression of fecal output.

Figure 1C shows that both (+)-TRV130 and morphine nearly eliminated colonic propulsion. Under baseline conditions, the artificial pellet was propelled 85 ± 3.5% of the total colon length within 10 min (569 ± 103 s: n=35). At 100 nM (+)-TRV130 or 1μM morphine, colonic propulsion was largely abolished over the 1 h period of recording. The spatiotemporal map (Figure 1D) constructed over a 10 min period shows the contractile pattern for the propulsion of the bolus (dark shade) in the Krebs solution, while it was stationary in the presence of TRV130 (100 nM) and morphine (1 μM).

Figure 2 shows the effects of acute and repeated 100 nM (+)-TRV130 on circular muscle contraction in colon (Fig. 2A) and ileum (Fig. 2B). Initial exposure to (+)-TRV130 produced robust contractions in both the colon and ileum. Repeated exposure to TRV130 continued to produce contractions of equal magnitude in the colon, but contractile responses diminished in the ileum, indicative of tolerance development in the ileum but not the colon.

Intracranial Self-Stimulation

The mean ± SEM maximum control rate (MCR) for all rats in the study was 63.6 ± 8.8 stimulations per trial, and the mean ± SEM control number of total stimulations delivered across all frequencies was 290.0 ± 84.4 stimulations per component. Figure 3 shows that (+)-TRV130 is the active isomer, and it produced both increases and decreases in ICSS (Fig. 3A,B). Doses of 0.1 and 0.32 mg/kg (+)-TRV130 produced small but significant increases in ICSS at one frequency (112 Hz), whereas a higher dose of 1.0 mg/kg (+)-TRV130 produced robust suppression of ICSS at all frequencies greater than 79 Hz. In analysis of the total number of stimulations per component, only ICSS depression produced by 1.0 mg/kg (+)-TRV130 was statistically significant, and this effect was blocked by pretreatment with 0.1 mg/kg naltrexone (Fig. 3B). Conversely, (−)-TRV130 at doses up to 10 mg/kg had no effect on ICSS (Fig. 3C,D). A dose of 10 mg/kg cocaine produced robust stimulation of ICSS in these rats (Fig. 3D).

Figure 4 shows the effects of cumulative treatment with (+)-TRV130 in 16 rats before initiation of repeated treatments with saline, (+)-TRV130 or morphine. Cumulative (+)-TRV130 produced dose-dependent changes in ICSS qualitatively similar to those observed when (+)-TRV130 doses were administered separately, although (+)-TRV130 was less potent when administered cumulatively. Thus, the initial dose of 0.32 mg/kg (+)-TRV130 produced weak but significant ICSS facilitation at two frequencies (79–89 Hz), and for this large group of rats, depression of ICSS at the highest frequency was also significant (158 Hz). Cumulative 1.0 mg/kg (+)-TRV130 also produced a biphasic effect, with ICSS facilitation at one frequency (89 Hz) and more prominent ICSS depression across a broad range of four high frequencies (112–158 Hz). Cumulative 3.2 mg/kg (+)-TRV130 robustly depressed ICSS at all frequencies above 70 Hz. In analysis of total stimulations per component, only the rate-decreasing effects of 3.2 mg/kg (+)-TRV130 reached the criterion for statistical significance.

Figure 5 shows the effects of cumulative (+)-TRV130 before and after repeated treatment with vehicle (Fig. 5A–C), 1.0 mg/kg/day (+)-TRV130 (Fig. 5D–F), or 3.2 mg/kg/day morphine (Fig. 5G–I). Left panels (A, D, G) show the same data as shown in Figure 4A, but now broken out into the three treatment groups. Across groups on Day 1, (+)-TRV130 produced similar dose-dependent decreases in high ICSS rates maintained by high brain-stimulation frequencies; however, increases in ICSS rates maintained by lower brain-stimulation frequencies were more variable across groups. ICSS facilitation on Day 1 was greatest in the Repeated Vehicle group and weakest in the Repeated Morphine group. Relative to these results on Day 1, repeated vehicle treatment did not significantly alter effects of (+)-TRV130. Conversely, both repeated 1.0 mg/kg/day (+)-TRV130 and repeated 3.2 mg/kg/day morphine attenuated the rate-decreasing effects and enhanced the ICSS rate-increasing effects of cumulative 0.32 and 1.0 mg/kg (+)-TRV130. After repeated (+)-TRV130 or morphine treatment, cumulative doses of 0.32 and 1.0 mg/kg (+)-TRV130 decreased ICSS at fewer frequencies and facilitated ICSS at more frequencies than on Day 1 (cf. Fig. 3 D and E and Fig. 3 G and H), and total stimulations per component also significantly increased on Day 9 relative to Day 1 (Fig. 3 F, I). However, repeated treatment with 1.0 mg/kg/day (+)-TRV130 or 3.2 mg/kg/day morphine did not alter the robust rate-decreasing effects produced by cumulative 3.2 mg/kg (+)-TRV130.

DISCUSSION

This study examined antinociceptive, constipating, and abuse-related effects of the G-protein-biased MOR agonist TRV130 in rodents. There were four main findings. First, TRV130 produced antinociception that was sustained during repeated dosing. This agrees with other evidence to suggest that MOR-mediated β-arrestin signaling contributes to tolerance in assays of acute pain-stimulated behavior, and that biased G-protein signaling may produce antinociception with reduced tolerance. Second, TRV130 also produced robust gastrointestinal inhibition in both in vivo and in vitro assays, and these effects were also resistant to tolerance. These results do not support the claim that gastrointestinal inhibition is mediated by β-arrestin signaling or that biased G-protein signaling might facilitate tolerance to gastrointestinal inhibition. Third, repeated TRV130 treatment also enhanced expression of abuse-related effects in an ICSS procedure, similar to effects of repeated morphine. This suggests that TRV130 has abuse potential similar to other MOR agonists, and that biased G-protein signaling does not protect against abuse liability during acute or chronic treatment. Lastly, all the above results were obtained with the (+)-isomer of TRV130, and ICSS studies indicated that the (−)-isomer is relatively inactive. Taken together, these results suggest that TRV130 is a stereoselective MOR agonist that may produce more sustained antinociception than morphine during chronic treatment, but it also produces morphine like adverse effects related to gastrointestinal inhibition and abuse liability.

Antinociception

The present results confirm and extend previous evidence to indicate that TRV130 produces antinociception in rodents and analgesic effects in humans (Chen et al. 2013; DeWire et al. 2013; Soergel et al. 2014a; Soergel et al. 2014b; Violin et al. 2014). To our knowledge, this is the first study to examine effects of repeated TRV130 treatment, and TRV130

antinociception was sustained under a regimen of repeated dosing that was sufficient to produce tolerance to morphine antinociception. A limitation to the present study is that tolerance was evaluated to only one treatment regimen of repeated TRV130 treatment, and different repeated dosing regimens (e.g. higher doses or longer treatment durations), or use of active but lower probe doses, may have produced evidence for antinociceptive tolerance. However, the apparent resistance of TRV130 antinociception to tolerance is consistent with both the lack of antinociceptive tolerance to another G-protein-biased MOR agonist (herkinorin; Lamb et al. 2012), and to evidence for reduced antinociceptive tolerance to morphine in β-arrestin 2 knockout mice (Raehal and Bohn 2011).

Gastrointestinal inhibition

Several recent findings have suggested that reductions in MOR-mediated β-arrestin signaling might reduce or eliminate the constipating effects of MOR agonists. First, morphine-induced constipation was reduced in β-arrestin 2 knockout mice relative to their wildtype controls (Raehal et al. 2005). Second, in studies of in vitro smooth muscle function, repeated morphine exposure produced tolerance to morphine effects in colons from β-arrestin 2 knockout mice but not from wildtype mice, suggesting that tolerance to any initial gastrointestinal inhibition might contribute to overall reduced constipation in β-arrestin knockouts (Kang et al. 2012). Lastly, a previous study in mice reported lower potency and effectiveness of TRV130 than morphine to produce constipation in mice. However, the present findings with TRV130 do not support a role for β-arrestin signaling in either initial expression of, or tolerance to, the constipating effects of MOR agonists. Acute TRV130 eliminated fecal output in vivo, and it was as effective as morphine to inhibit colonic propulsion and stimulate colonic and ileal circular muscle contractions in vitro (present study, Kang, 2012 #3266;Ross, 2008 #3267}. Tolerance developed to TRV130 effects on contraction of ileal circular muscle as it does with morphine (Ross et al. 2008), but tolerance did not develop to other effects of TRV130 on colonic circular muscle contraction, colonic propulsion, or in vivo fecal output. Although different repeated dosing regimens, or use of active but lower probe doses, may have produced evidence for tolerance to these other effects, the present results nonetheless suggest that, as with morphine, tolerance develops more readily to TRV130 effects on ileum than on colon. Overall, the profile of acute and repeated TRV130 administration on these measures of gastrointestinal function did not differ from the effects of morphine. Moreover, this preclinical evidence for robust constipating effects of TRV130 agrees with clinical evidence to suggest that TRV130 produces constipation equivalent to that of morphine in humans (Viscusi et al. 2016). Reasons for the discrepancy in results between the present and previous studies remain to be determined. One possibility is that TRV130 lacks sufficient bias for G-protein vs. β-arrestin signaling to produce reliable decreases in gastrointestinal inhibition, but evaluation of this hypothesis will require new compounds with greater G-protein bias.

Abuse liability

(+)-TRV130 effects on ICSS were similar to effects reported previously for morphine and other MOR agonists (Altarifi and Negus 2011; Altarifi et al. 2013; Miller et al. 2015), and as such, these results suggest qualitatively similar abuse liability for (+)-TRV130 and other MOR agonists. Thus, MOR agonists such as morphine produce a mixed profile of effects on

rates of ICSS that includes both increases in low ICSS rates maintained by low brain-stimulation frequencies and decreases in high ICSS rates maintained by high brain-stimulation frequencies. In opioid-naïve subjects, rate-decreasing effects of MOR agonists predominate. These rate-decreasing effects oppose and limit expression of abuse-related rate-increasing effects, and expression of abuse-related ICSS facilitation is typically weak or absent. However, repeated MOR agonist treatment produces tolerance to rate-decreasing effects and enhanced expression of abuse-related ICSS facilitation.

In agreement with this profile for other MOR agonists, acute treatment with single or cumulative (+)-TRV130 doses in opioid-naïve rats produced a mixed profile that included weak ICSS facilitation at low doses and more pronounced ICSS depression at higher doses. As with other abuse-related MOR agonist effects (e.g. Shannon and Holtzman 1976), TRV130 effects on ICSS were stereoselective, and rate-decreasing effects of (+)-TRV130 were blocked by naltrexone, providing additional support for a role of MORs in mediating (+)-TRV130 effects. Also like other MOR agonists, repeated treatment with (+)-TRV130 produced tolerance to rate-decreasing effects and enhanced expression of abuse-related ICSS facilitation. Lastly, expression of ICSS facilitation by (+)-TRV130 was also enhanced by repeated treatment with a morphine dosing regimen sufficient to enhance ICSS facilitation by morphine and other MOR agonists [Altarifi, 2013 #2625;Miller, 2015 #3264}. Insofar as drug effects on ICSS are predictive of abuse liability, these results suggest that (+)-TRV130 has abuse liability similar to that of morphine and other MOR agonists.

This evidence for abuse liability by (+)-TRV130 contrasts with a recent report that a single dose of (±)-TRV130 failed to produce a conditioned place preference in mice (Manglik et al. 2016). However, the present ICSS results are consistent with preclinical evidence that MOR agonist rewarding effects are retained and enhanced in β-arrestin2 knockout mice (Bohn et al. 2003), and evidence from clinical studies that TRV130 produces a morphine-like increase in abuse-related subjective effects, such as scores of “Good Effects,” “Liking,” and “High.” (Soergel et al. 2014a).Thus, the preponderance of evidence suggests that G-protein-biased mu agonists like TRV130 will retain opioid-like abuse potential.

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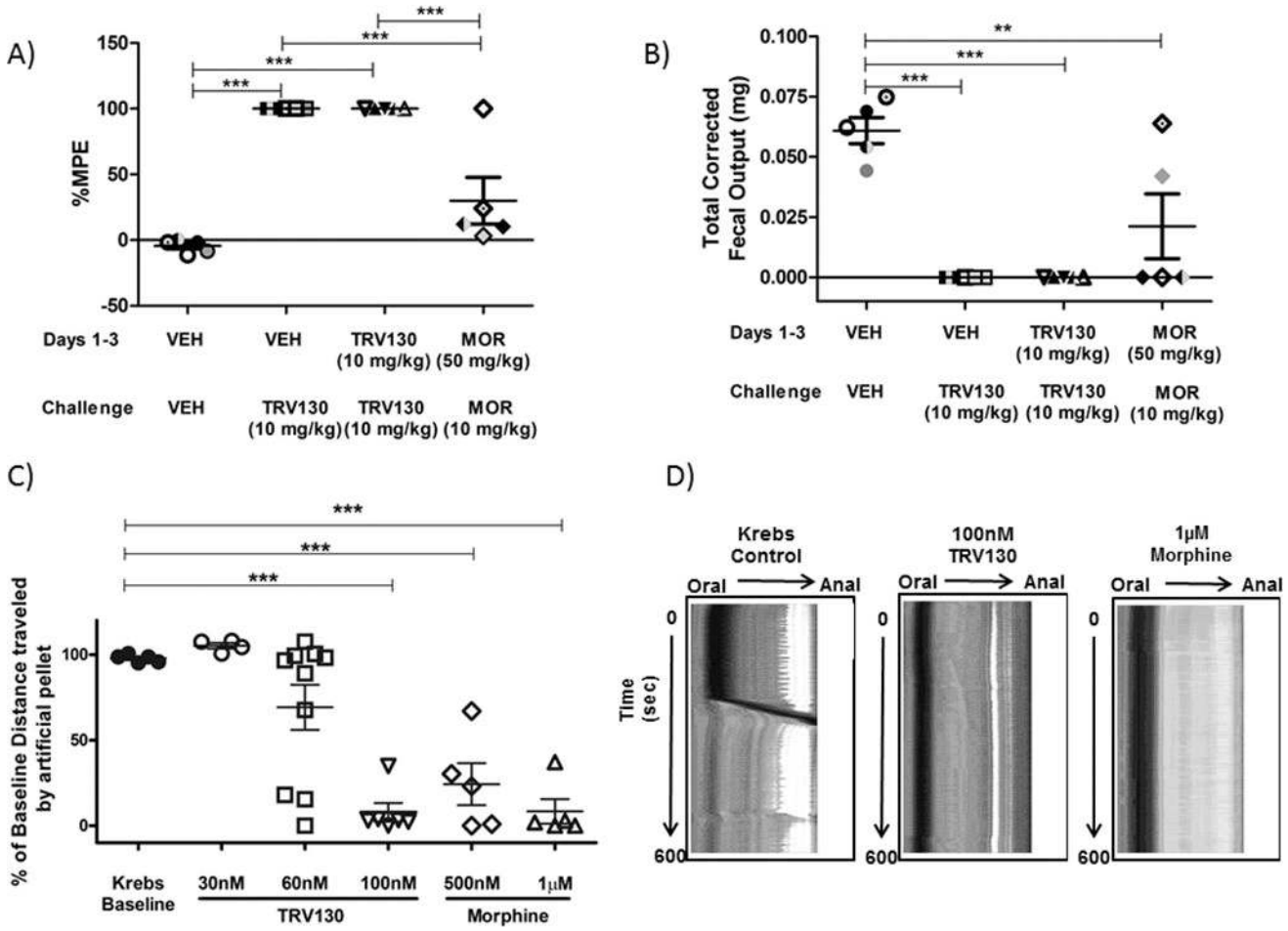

Figure 1.

Comparison of effects produced by (+)-TRV130 and morphine on in vivo antinociception and fecal output (A,B) and in vitro colonic propulsion (C,D). In Panels A and B, abscissae show treatments, and ordinates show measures of antinociception (percent maximum possible effect, %MPE, Panel A) or fecal output over 60 min (expressed as a fraction of food consumption over the previous 24h; Total Corrected Fecal Output, Panel B). Bars show mean ± SEM in 5 mice. Asterisks indicate a significant difference between the designated treatments as indicated by a significant one-way ANOVA in Panel A [$F(3,16)=33.78$, $P<0.0001$] and Panel B [$F(3,16)=15.74$, $P<0.0001$] followed by Bonferroni's post hoc test (**, $P<0.01$; ***, $P<0.001$). In Panel C, the abscissa shows treatment, and the ordinate shows distance traveled by an artificial pellet (expressed as a percentage of baseline pellet propulsion in the presence of Krebs solution for each colon). Bars show mean ± SEM in 5–10 colons. Asterisks indicate a significant difference from Krebs Baseline as indicated by a significant one-way ANOVA [$F(5,29)=14.01$, $P<0.0001$] followed by Bonferroni's post hoc test (***, $P<0.001$). Panel D shows representative spatiotemporal maps from a 10 min video recording of colon inserted with an artificial pellet (black band). Distance along Y axis indicates time and the oral to anal gradient is represented on the X-axis. Note the absence of pellet propulsion in the presence of (+)-TRV130 (100 nM) and morphine (1 uM). Each symbol represents the same mouse in the two assays.

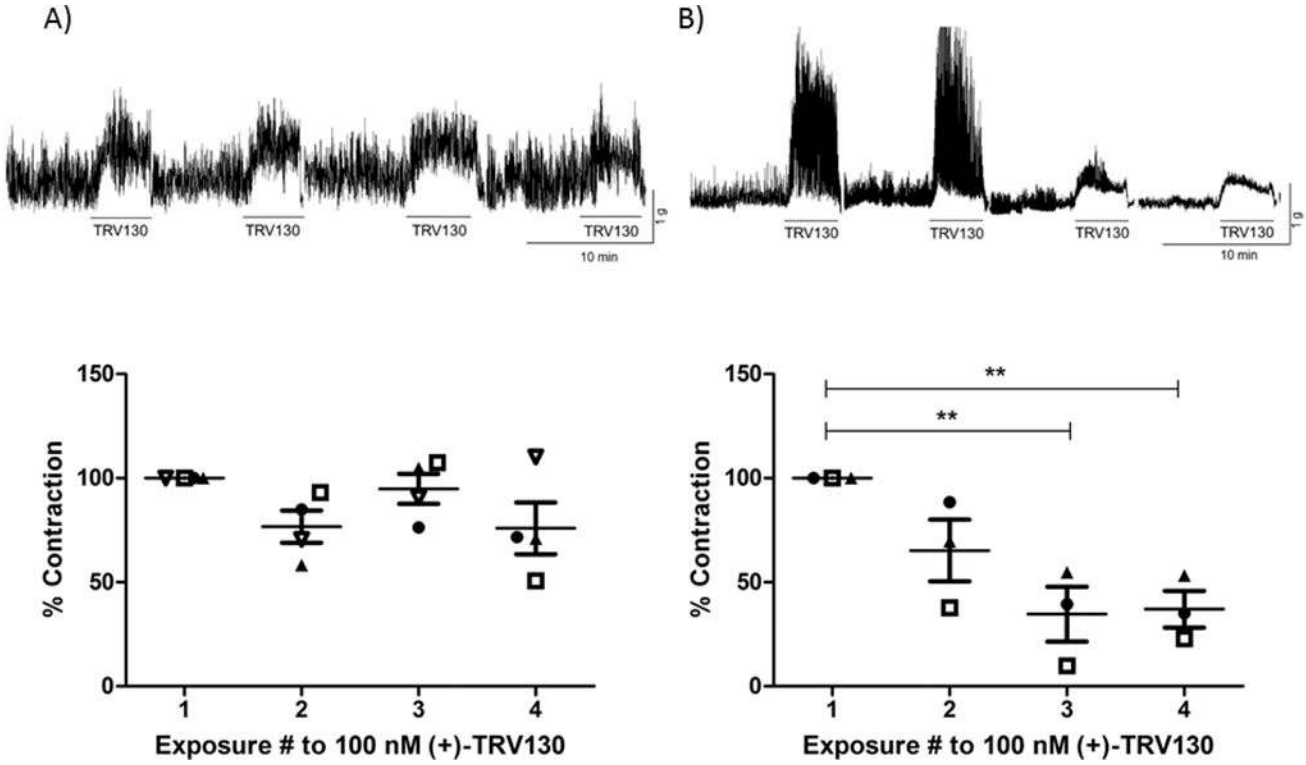

Figure 2.

Circular muscle contractions elicited by repeated treatment with 100 nM (+)-TRV130 treatment in mouse colon (A; N=4) and ileum (B; N=3). Abscissae: Exposure number (4 sequential exposures). Ordinates: Percent of contraction elicited by the initial exposure (% Contraction). Representative raw traces are shown above each panel. Bars show mean ± SEM, and points show data for individual experiments. Asterisk indicates significant difference from contraction during exposure 1 determined by repeated measures ANOVA in Panel B [$F(3,6)=14.72$, $P<0.01$] followed by Bonferroni's post hoc test (**, $P<0.01$). The ANOVA in Panel A was not significant [$F(3,9)=1.875$, $P=0.20142$].

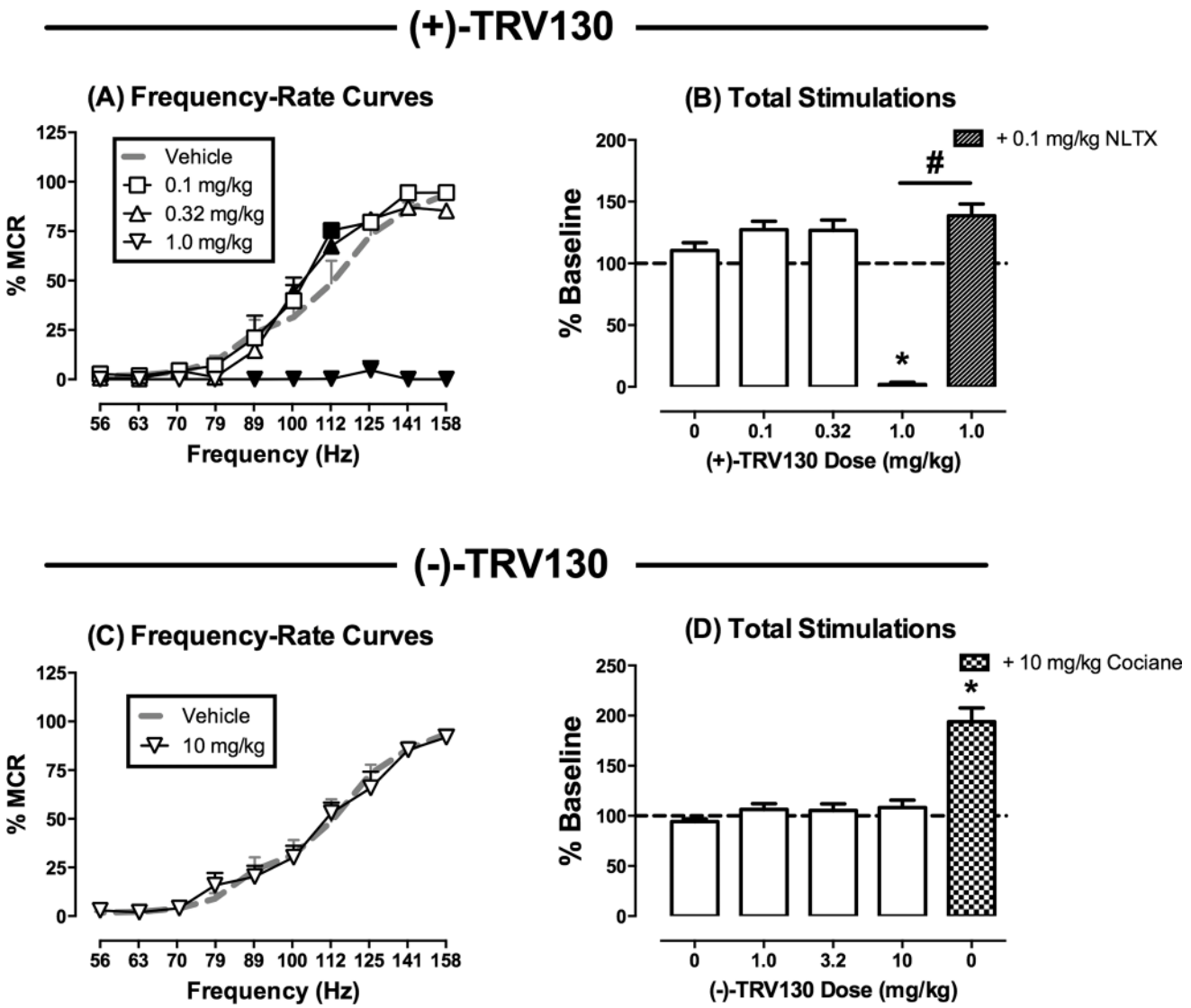

Figure 3.

Effects of (+)-TRV130 (A,B) and (−)-TRV130 (C,D) on ICSS in rats. Left panels (A,C) show ICSS frequency-rate curves. Abscissae: frequency of electrical brain stimulation in hertz (log scale). Ordinates: ICSS rate expressed as percent maximum control rate (%MCR). Filled symbols indicate frequencies at which ICSS rates were significantly different from those observed after vehicle administration. Only the highest dose of (−)-TRV130 is shown in Panel C for clarity. Right Panels (B,D) show summary data for the total number of stimulations per test component expressed as a percentage of total pre-test baseline stimulations. Panel B also shows effects of 0.1 mg/kg naltrexone (NLTX) pretreatment on depression of ICSS produced by 1.0 mg/kg (+)-TRV130 (compared with effects 1.0 mg/kg (+)-TRV130 alone by t test), and Panel D also shows effects of 10 mg/kg cocaine (compared with effects of vehicle by t-test). Abscissae: dose of drug. Ordinates: percent control stimulations per test component. * Asterisks indicate significant effect compared to vehicle. # Number sign indicates significantly different from 1.0 mg/kg (+)-TRV130 alone. Statistical results were as follows (only interaction results are reported for two-way

ANOVAs): (A) significant dose × frequency interaction [F(27,135)=23.45; P<0.0001]. (B) Significant effect of (+)-TRV130 dose [F(3,15)=117.0; P<0.0001]; significant effect of naltrexone (t=14.1, P<0.0001). (C) No significant dose × frequency interaction. (D) No significant main effect of (−)-TRV130 dose; significant effect of cocaine (t=4.8, P=0.005). All points show mean ± SEM for 6 rats.

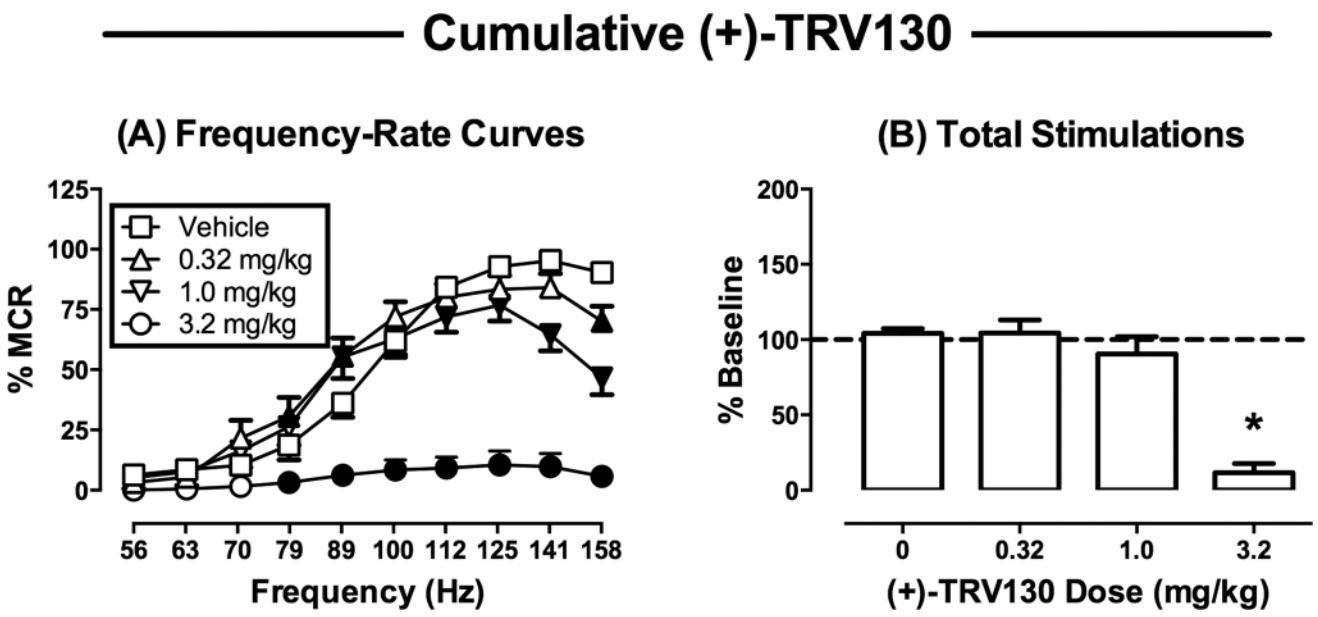

Figure 4.

Effect of cumulative (+)-TRV130 on ICSS in rats. For this study, (+)-TRV130 was administered by cumulative dosing in a single test session rather than as separate doses on different test days. For description of axes and symbols, please refer to Fig. 1. ANOVA results were as follows (only interaction results are reported for the two-way ANOVA): (A) Significant dose × frequency interaction [$F(27,405)=21.3$; $P<0.0001$]. (B) Significant effect of dose [$F(3,45)=53.5$; $P<0.0001$]. All points show mean ± SEM for 16 rats.

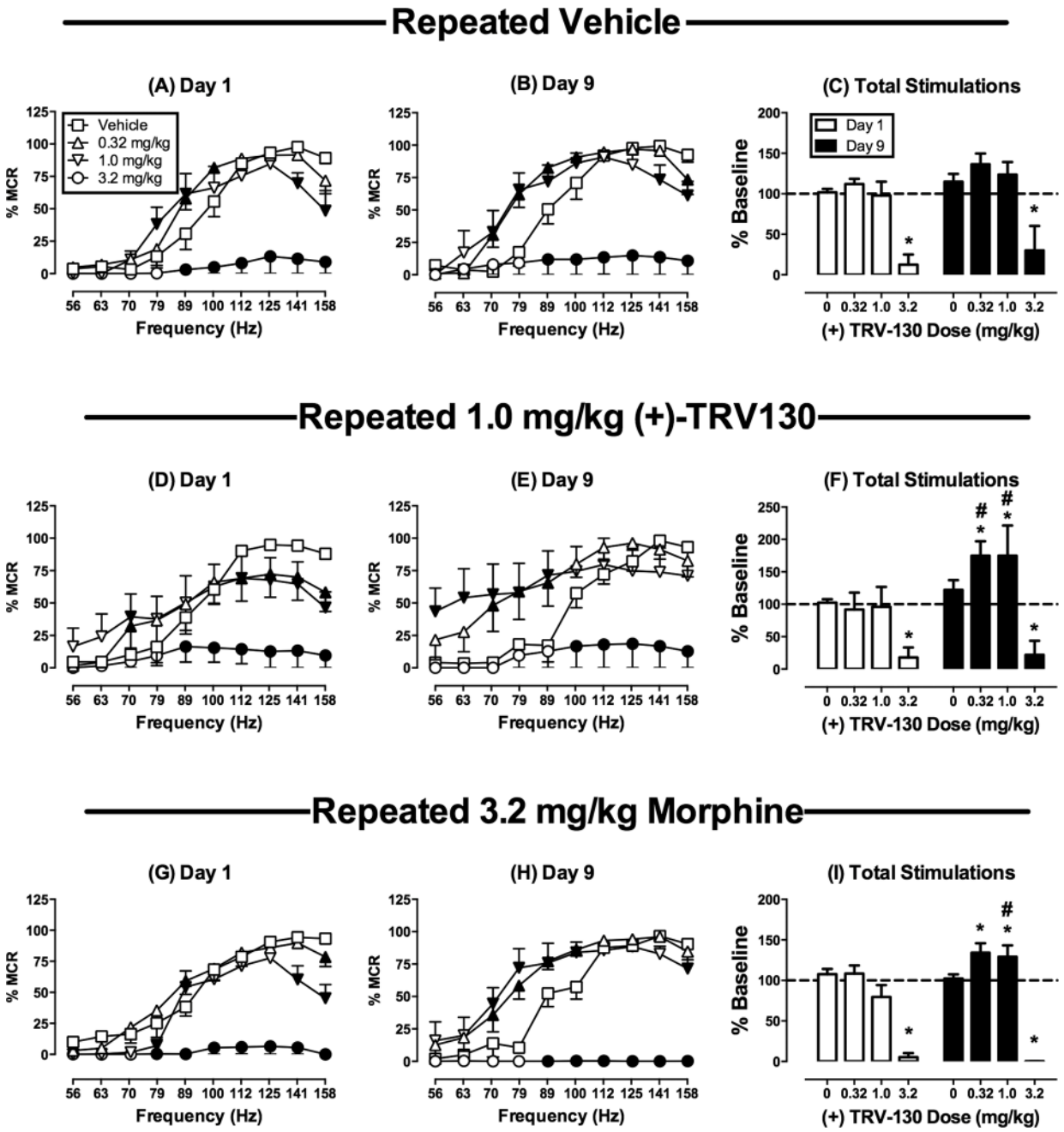

Fig. 5.

Modulation of (+)-TRV130 effects on ICSS after repeated treatment with vehicle, 1.0 mg/kg/day (+)-TRV130, or 3.2 mg/kg/day morphine. Subjects were injected with saline (A,B,C), 1.0 mg/kg/day (+)-TRV130 (D,E,F), or 3.2 mg/kg/day morphine (G,H,I) for 7 days, and the effect of cumulative doses of (+)-TRV130 on ICSS was determined before and after repeated administration. For description of axes and symbols, please refer to Figure 1. * Asterisks indicate significant effect compared to within-day vehicle, whereas # number signs indicate significant effect between days for the same dose. ANOVA results were as follows: (A) Significant dose × frequency interaction [$F(27,108)=7.9$; $P<0.0001$]. (B) Significant

dose × frequency interaction [F(27,108)=13.4; P<0.0001]. (C) No significant dose × day interaction, but a significant main effect of dose [F(3,12)=36.8; P<0.0001]. (D) Significant dose × frequency interaction [F(27,108)=6.0; P<0.0001]. (E) Significant dose × frequency interaction [F(27,108)=3.6; P<0.0001]. (F) Significant dose × day interaction [F(3,12)=5.4; P=0.0137]. (G) Significant dose × frequency interaction [F(27,135)=10.7; P<0.0001]. (H) Significant dose × frequency interaction [F(27,135)=12.5; P<0.0001]. (I) Significant dose × day interaction [F(3,15)=5.2; P=0.0113]. All points show mean ± SEM for 5–6 rats.