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The G-protein biased mu-opioid agonist, TRV130, produces reinforcing and antinociceptive effects that are comparable to oxycodone in rats.

C. Austin Zamarrripa¹, Shelley R. Edwards², Hina N. Qureshi², John N. Yi¹, Bruce E. Blough³, and Kevin B. Freeman¹

¹Department of Psychiatry and Human Behavior; University of Mississippi Medical Center, Jackson, MS 39216 USA

²Department of Psychology and Neuroscience, Millsaps College, Jackson MS, 39210 USA

³Center for Drug Discovery, Research Triangle Institute, Research Triangle Park, NC 27709 USA

Abstract

Mu-opioid agonists (e.g., oxycodone) are highly effective therapeutics for pain. However, they also produce reinforcing effects that increase their likelihood of abuse. Recent strategies in drug development have focused on opioids with biased receptor-signaling profiles that favor activation of specific intracellular pathways over others with the aim of increasing therapeutic selectivity. TRV130, a mu-agonist biased towards G-protein signaling, produces antinociceptive effects comparable to the mu-agonist, morphine, but exhibits reduced side effects. However, in terms of abuse potential, we know of no published preclinical data investigating the effects of TRV130 as a reinforcer. In the present study, we assessed the relative reinforcing effects of TRV130 and oxycodone, a commonly-prescribed mu-agonist, in rats self-administering the drugs under a progressive-ratio (PR) schedule of reinforcement. In addition, we assessed the relative potency and efficacy of TRV130 and oxycodone in rats in a test of thermal antinociception (Hot Plate). For self-administration, male Sprague-Dawley rats (n=7) self-administered intravenous infusions of TRV130 or oxycodone (0.01–0.32 mg/kg/inj) under a progressive-ratio (PR) schedule of reinforcement. For the Hot-Plate Test, male rats (n=7) received subcutaneous injections of TRV130 (0.1–3.2 mg/kg/inj) or oxycodone (0.1–5.6 mg/kg/inj), and nociceptive response latencies

Correspondence: C. Austin Zamarrripa, Department of Psychiatry and Human Behavior, The University of Mississippi Medical Center 2500 N. State Street, Jackson, MS 39216, Phone: (601) 815-5622, czamarippa@umc.edu.

Authors Disclosures

Contributors

Kevin Freeman and Austin Zamarrripa designed the study. Austin Zamarrripa oversaw the conduct of the research and the collection and management of data. Austin Zamarrripa and John Yi conducted the self-administration study. Shelley Rae Edwards and Hina Qureshi conducted the Hot-Plate study. Bruce Blough synthesized and supplied the TRV130 for the study. All authors participated in compiling and reviewing relevant literature. Austin Zamarrripa analyzed the data. Austin Zamarrripa and Kevin Freeman wrote the first draft and the revised draft of the manuscript. All authors participated in the editing of the first and revised drafts. All authors read and approved the final manuscript.

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Conflict of Interest

No conflict declared.

were measured. TRV130 and oxycodone were equi-potent and equi-effective in selfadministration and thermal antinociception. This study demonstrates that TRV130 produces reinforcing and antinociceptive effects that are quantitatively similar to oxycodone, and that a biased-signaling profile does not necessarily reduce abuse potential.

Keywords

Biased Agonists; TRV130; Oxycodone; Self-Administration

1. Introduction

Opioid agonists (e.g., oxycodone and morphine) are highly efficacious for the treatment of pain. However, their therapeutic effects are coupled with adverse side effects that include tolerance, dependence, respiratory depression, and abuse liability (Chan 2017). Opioid agonists mediate their physiological actions through the G protein-coupled receptor (GPCR) family of opioid receptors, specifically the mu-opioid receptor (Kieffer, 1999; Matthes et al., 1996; Mello and Negus 1996). It is well established that GPCRs modify cell activity through various intracellular pathways including G-protein and b-arrestin signaling (Bologna et al., 2017), two pathways that are known to be prominent mediators of mu-opioid agonist effects (Bohn et al., 2004).

Recently, there has been increased interest in distinguishing the signaling pathways responsible for both therapeutic effects and unwanted side effects of drugs with the aim of identifying optimal signaling profiles that increase therapeutic selectivity while decreasing unwanted side effects (Dewire et al., 2013; Manglik et al., 2016; Varadi et al., 2016). As such, agonists of various receptors are being developed that preferentially activate certain pathways over others, a process which has been termed functional selectivity or biased agonism (Kenakin, 2003; Rankovic et al., 2012). Relevant to mu-opioid receptor function, studies have determined that the therapeutic effects may, to some degree, be separated from certain side effects through signaling selectivity. For example, b-arrestin knock-out (KO) mice, when treated with morphine, demonstrated less respiratory depression but equivalent antinociception compared to wild-types, and tolerance to morphine's antinociceptive effects was reduced in the KOs (Bohn et al. 1999; Bohn et al. 2000; Bohn et al. 2002; Raehal et al. 2005). However, b-arrestin KO mice, when treated with morphine, exhibit dependence and morphine-induced reward in conditioned place preference tests similar to wild-type mice (Bohn et al. 2000; Bohn et al. 2003). Together, these studies indicate that b-arrestin may be responsible for some, but not all, of the side effects produced by mu-opioid agonists.

Several biased mu-agonists have been developed that produce relatively selective G- protein signaling with minimal b-arrestin activity. Mitragynine pseudoindoxyl, PZM21, and TRV130 have all demonstrated robust antinociceptive properties and reduced respiratory depression relative to standard mu-agonists in rodent models (Chen et al. 2013; Cheng et al., 2018; DeWire et al. 2013; Gan et al., 2017; Manglik et al. 2016; Varadi et al. 2016). Of these three novel compounds, only TRV130 has progressed to clinical trial (Singla et al. 2017; Siuda et al., 2016; Soergel et al. 2014a; Soergel et al. 2014b; Viscusi et al. 2016). When

administered to healthy volunteers, TRV130 produced less nausea and milder respiratory depression when compared to morphine (Soergel et al. 2014a; Soergel et al. 2014b). In patients with moderate to severe acute pain, TRV130 produced antinociceptive effects at a faster rate than morphine and produced less aversive side effects such as gastrointestinal inhibition and tolerance (Singla et al. 2017; Viscusi et al. 2016).

While the data above are encouraging, the data available on the abuse-related effects of TRV130 suggest that it will have abuse liability. In mice, a single antinociceptive dose of TRV130 failed to produce a place preference (Manglik et al., 2016), suggesting that doses needed to treat pain might not produce abuse-related effects. However, TRV130 produced subjective effects similar to morphine in healthy human volunteers, indicating that the stimulus effects of biased mu- agonists are similar to standard prescription opioids (Soergel et al. 2014a). In a model of intracranial self-stimulation (ICSS) in rats, TRV130 demonstrated effects on rates of ICSS similar to that of morphine, indicating that TRV130 produces reward-enhancing effects that are consistent with commonly-abused opioids (Altarifi et al. 2017).

When taken together, studies thus far indicate that TRV130 (and perhaps other G-protein-biased mu-opioid receptor agonists) is qualitatively-similar to other mu-opioid medications in terms of abuse liability. However, key quantitative comparisons of TRV130 and standard mu- agonist therapies have yet to be established, chiefly through an evaluation of TRV130 as a reinforcer in drug self-administration which is the gold standard approach for characterizing the abuse of potential of drugs (Ator and Griffiths, 2003). While we may infer that TRV130 will function as a reinforcer based on the evidence discussed above, we do not know its potency as a reinforcer relative to any mu-opioid agonist, nor we do know its relative reinforcing efficacy (i.e., maximum reinforcing effect). Therefore, we compared TRV130 as a reinforcer to oxycodone, a commonly-prescribed opioid therapeutic, in rat self-administering the drugs under a progressive- ratio (PR) schedule of reinforcement. Furthermore, based on results showing that functionally- equivalent antinociceptive doses of TRV130 and morphine produced disparate effects in conditioned place preference (Manglik et al., 2016), the second part of the study compared the thermal antinociceptive effects of TRV130 to oxycodone with the general hypothesis that the antinociceptive potency of TRV130 relative to oxycodone would be greater than its relative potency as a reinforcer, an outcome that would indicate a larger “therapeutic window” for TRV130.

2. Method

2.1 Subjects

Male Sprague-Dawley rats (Envigo Laboratories, New Jersey, USA) arrived at the facility at approximately 65 days of age and weighed 275–299 g on arrival. All rats were acclimated to their housing chambers for at least one week prior to the initiation of studies. Subjects were pair housed with *ad libitum* access to food and water and were maintained on a reversed 12-h light/dark cycle (lights off at 0800) with experiments occurring during the dark phase. Experiments were conducted in compliance with the National Research Council’s Guide for Care and Use of Laboratory Animals (2011) and approved by the University of Mississippi Medical Center’s Institutional Animal Care and Use Committee.

2.2 Surgery

Subjects in the self-administration study received intravenous catheters implanted into their right jugular veins as previously described (Townsend et al., 2017). Catheters were guided subcutaneously to a vascular access port (Instech Laboratories, Plymouth Meeting, PA, USA) that was implanted subcutaneously behind the shoulder blades. Subjects received carprofen (5.0 mg/kg s.c.) once pre-operatively and twice post-operatively (24 and 48 h following surgery) for analgesic support. For the 7-day post-surgical period, catheters were infused daily with gentamycin (0.08 mg, i.v.) to prevent infection and heparinized saline (30 U/ml, i.v.) to facilitate patency. Subjects were allowed to recover for 7-days prior to self-administration sessions. Once testing began, catheters were flushed daily with heparinized saline after each self-administration session. Every 14 days and at the conclusion of the study, patency was verified by intravenous infusions of Brevital (5 mg/kg i.v.), followed by 0.1 ml of heparinized saline. Catheters were considered patent if ataxia was apparent within 3 sec. If catheters were considered non-functional, a new catheter was implanted into the left jugular vein, and the subject continued in the experiment. Postoperative care after implantation of a second catheter was identical to that described above.

2.3 Apparatus

2.3.1 Operant Chambers.—Eight operant test chambers (Med-Associates, St. Albans, VT, USA) were used during the self-administration procedure. Self-administration sessions occurred daily and began at approximately 0800. Operant chambers were equipped with two retractable levers and corresponding white stimulus lights mounted above each lever. Drug infusions were delivered at a rate of 0.018 ml/s from 10 ml plastic syringes that were seated in infusion pumps outside of the operant box (Razel Scientific, St. Albans, VT, USA). Drugs were delivered through polyethylene tubing that was connected to the plastic syringe and threaded through a spring leash (Instech Laboratories) that connected to the vascular access port located on the subject's back. A PC equipped with Med-Associates software (Med-PC for Windows; St. Albans, VT, USA) controlled experimental conditions and recorded data.

2.3.2 Hot-Plate Apparatus.—A Hot-Plate analgesiometer (Omnitech Electronics, Columbus, OH, USA) with a plastic enclosure (11" L, 11" W, 8" H) was used to test the thermal antinociceptive properties of the drugs. The hot plate was maintained at 52.5°C during each experimental session. Latencies to emit a nociceptive response were recorded by a blinded observer with a digital timer.

2.4 Drugs

Oxycodone hydrochloride was generously provided by the National Institute on Drug Abuse. TRV130 hydrochloride was synthesized and provided by Dr. Bruce Blough at the Research Triangle Institute (Research Triangle Park, NC, USA). All drugs were prepared in 0.9% sterile saline and doses are expressed as the salt.

2.5 Procedures

2.5.1 Oxycodone Self-Administration Training.—Subjects ($n = 7$) were trained to self-administer oxycodone (0.056 mg/kg/inj) on a fixed-ratio 1 (FR1) schedule of

reinforcement. The training dose was selected based on previous work showing it to be reliable for the acquisition of self-administration (Townsend et al., 2017). The maximum number of infusions for this phase was set at 20, with the session running 2h or until 20 infusions occurred. The response ratio was increased to FR5 once a subject met one of the following criteria: a) 3-consecutive days of 15 or more infusions, b) 2-consecutive days of 20 infusions, or c) after 14-days of self-administration at FR1 without reaching either of the previous two criteria. Subsequently, self-administration under the FR5 schedule of reinforcement was maintained until one of the following criteria were met: a) 2-consecutive days of 20 infusions, or b) 3-consecutive days of 15 or more infusions. Failure to meet one of these criteria resulted in removal from the study. Notably, all subjects that met the criteria under the FR5 schedule did so by self-administering the total 20 injections of 0.056 mg/kg/inj oxycodone for 2-consecutive sessions. As such, the less stringent 3-day criterion with fewer injections was not applied.

Once subjects completed training under the FR schedule, self-administration of the training dose of oxycodone (0.056 mg/kg/inj) on a PR schedule of reinforcement began. Session time increased to 4h with no limit on infusions earned. The response-cost series was derived from a logit equation previously described by Thomsen et al., (2005): $19 \times [1 + \log(\text{step}/7 - 0.3 \times \text{step})]$, rounded to the nearest integer up to step 23, after which the response cost required to receive a reinforcer was increased linearly by 12, resulting in the following response-cost values: 3, 9, 13, 16, 18, 20, 22, 24, 25, 27, 28, 29, 31, 32, 34, 35, 37, 39, 41, 44, 47, 52, 64, 76, 88, and so forth. Training was considered complete when the following stability criteria were met: a) infusions earned across 3 days were within 20% of the 3-session mean, and b) there were no upward or downward trends.

Once self-administration was stable under the PR schedule, oxycodone was removed as a response contingency and replaced with saline infusions. Training under this condition for each subject was considered complete once the following criteria were met: a) infusions earned across 3 days were within 20% of the 3-session mean, b) there were no upward or downward trends, and c) saline infusions earned were at least half the mean number of oxycodone infusions earned in the PR training phase.

2.5.2 Oxycodone Versus TRV130 Self-Administration.—Each subject completed a dose series (0.01, 0.032, 0.1, 0.32mg/kg/inj) for oxycodone and TRV130 under the PR schedule described above. This dose series was selected based on results from our previous work studying oxycodone as a reinforcer in rats (Townsend et al., 2017). All test conditions were administered in a counterbalanced order for both drug (oxycodone and TRV130) and dose across all subjects such that the series of conditions for each subject varied non-systematically by both dose and drug. Responding for each test condition was considered stable if a) infusions earned across 3 days were within 20% of the 3-session mean, and b) there were no upward or downward trends in infusions earned. A saline test point was included within the counterbalanced test sequence for all subjects as a non-reinforcer control.

After each completed test of a drug condition and previous to the subsequent test, the training dose of oxycodone (0.056 mg/kg/inj) was offered for one session on the PR

schedule to monitor the stability of responding for the training dose over the time period required to test all drug conditions, and this “return to baseline” session was followed by a series of saline sessions sufficient to reduce responding to within 20% of each subject’s pre-test saline-infusion baseline. Notably, the mean oxycodone infusions for each of these sessions were within 20% of the pretest baseline-infusion mean for all subjects with no systematic direction of change, and all subjects decreased responding to within 20% of their pre-test saline levels within 3-sessions of saline availability. As such, all test conditions were conducted within a context of comparable baseline performance.

2.5.3 Thermal Antinociception.—Thermal antinociception was tested in a separate group of rats. On Day 1 of the study, each subject (n=7) was placed on the unheated apparatus for an acclimation period of 60 sec before being removed from the hot-plate and returned to their home cage. Following this acclimation exposure, the surface of the apparatus was heated to 52.5°C. Prior to placement on the hot plate, each subject received a subcutaneous (s.c.) injection of saline, followed by a test of nociception occurring after a 15-min interval. Immediately following the test of nociception, the subject received another saline injection before being placed on the hot plate again 15-min later for the second test of nociception. This injection-test series was repeated for a total of 5 tests to acclimate the subjects to the cumulative-dosing design (described below). Notably, all subjects were removed from the hot plate immediately following a nociceptive response (paw lick or lift not related to ambulation).

For experimental sessions, each subject received a single saline injection followed 15 min later by placement on the hot plate heated to 52.5°C. Subjects were removed from the hot plate immediately after emitting a nociceptive response, defined as a paw lick or paw lift not related to ambulation, and the latency (in seconds) to emit the response was recorded. If 60 sec passed without a nociceptive response, subjects were removed from the hot plate. Following the first test of nociception, subjects received increasing doses of TRV130 or oxycodone with tests of nociception between each injection. For drug injections, each subject received all doses of a particular drug in a single session in a cumulative-dosing approach that rendered a dose range in half-log intervals that encompassed a full range effect (<20% to >80% maximum possible effect). Oxycodone and TRV130 were administered in a counterbalanced order, such that half of the subjects received oxycodone first and half the subjects received TRV130 first. Tests of nociception occurred 15 min after each injection and were followed immediately after by the next injection in the cumulative-dose series. An experimenter blinded to the subjects’ conditions recorded response latencies while another experimenter administered injections. Test sessions were separated by a 72-h interval.

2.6 Data Analysis

2.6.1 Self-Administration.—Dose-response relations for oxycodone and TRV130 were analyzed with a two-way ANOVA with the within-subject factors of Drug (oxycodone or TRV130) and Dose. Dunnett’s multiple-comparisons tests were used to compare mean infusions between drugs at a dose and to compare each dose of each drug to saline. Relative potencies of TRV130 and oxycodone were quantified by fitting the ascending limbs of the aggregated dose- response functions, and ED50 values and 95% confidence intervals were

calculated via linear regression (GraphPad Prism 7.0) ED50 values were considered to be significantly different if the 95% confidence intervals did not overlap.

2.6.2 Thermal Antinociception.—Hot-plate latencies were expressed as % maximum possible effect (%MPE) according to the following equation: $\%MPE = [(Test\ Latency - Saline\ Latency) / (60 - Saline\ Latency)] \times 100$ where the test latency was the latency for the rat to emit a nociceptive response after administration of a dose of a drug, and saline latency was the latency to emit a response following the initial saline injection. Individual %MPE for each drug condition was plotted as a function of logarithmic dose. %MPE dose-effect functions spanned a range that included $\pm 20\%$ and $\pm 80\%$ effect. To compare relative potencies of TRV130 and oxycodone, the dose-response data were aggregated across subjects and fit with linear regression to determine ED50 values and 95% confidence intervals as described for the self-administration study above. ED50 values were considered to be significantly different if the confidence intervals did not overlap.

3. Results

Figure 1 illustrates the average self-administered infusions of oxycodone or TRV130 across subjects as a function of dose. All rats completed all dose conditions. A repeated-measures ANOVA revealed a main effect of Dose ($F[4,24] = 26.1$; $P < 0.0001$) but no main effect of Drug ($F[1,6] = 0.7$; $P = 0.4332$) or Dose $\times$ Drug interaction ($F[4,24] = 2.5$; $P = 0.0672$). Both oxycodone and TRV130 functioned as reinforcers in a dose-dependent manner, with all but the lowest dose of each drug resulting in injections significantly higher than saline (all p 's < 0.05). There were no significant differences between injections of oxycodone and TRV130 at any dose (all p 's > 0.05). Furthermore, there was no significant difference in the ED50 values for oxycodone and TRV130 (i.e., confidence intervals overlapped, see Table 1), indicating that they were equipotent as reinforcers.

Figure 2 illustrates the thermal antinociceptive effects of oxycodone and TRV130 aggregated across subjects in the Hot-Plate test. %MPE values are plotted as a function of dose for each drug. Both oxycodone and TRV130 produced full antinociception (i.e., 100% MPE) at the highest doses tested for each drug. ED50 values for oxycodone and TRV130 were not significantly different (i.e., confidence intervals overlapped, see Table 1), indicating that the drugs were equipotent at producing thermal antinociception under the current conditions.

4. Discussion

The objective of the current study was to quantitatively compare the reinforcing and antinociceptive effects of the biased μ -agonist, TRV130, to oxycodone in rats. The novel finding from this study was that TRV130 and oxycodone were equipotent and equieffective as reinforcers when self-administered by rats under a PR schedule of reinforcement. Consistent with previous findings (Altarifi et al., 2017; Dewire et al., 2013; Manglik et al., 2016), TRV130 produced robust thermal antinociception in rats and was equipotent to oxycodone on this endpoint. Together, these results suggest that TRV130 will be comparable to oxycodone and other standard prescription opioids in terms of abuse- and therapeutic-related effects.

To our knowledge, this is the first publically-available report to investigate TRV130 as a reinforcer. However, the self-administration results are consistent with previous reports of TRV130 producing abuse-related effects. For example, when administered before an ICSS test in rats, lower doses of TRV130 produced rate-increasing effects, and higher doses produced rate- decreasing effects, a typical profile for common mu-opioid agonists (e.g., morphine; Altarifi et al., 2017; Altarifi and Negus, 2011; Koob et al., 1975). Furthermore, in human subjects, TRV130 produced positive subjective effects comparable to morphine (Soergel et al., 2014b). On the other hand, a recent study reported that TRV130 and PZM21, another mu-agonist biased towards G- protein signaling, did not produce place preferences at doses sufficient for producing thermal antinociception (Manglik et al., 2016). Results such as these have generated cautious optimism that biased mu-agonists will have reduced abuse potential relative to standard opioid therapeutics (e.g., see Volkow and Collins, 2017).

We submit that the degree of optimism warranted for biased mu-agonists depends largely on a therapeutic index that relates their antinociceptive potency to abuse-related potency. Drugs with high potency for antinociception and low potency for abuse-related effects could be prescribed at doses that do not produce reinforcing effects, which in theory should decrease risk for abuse. Manglik et al. (2016) only tested single doses of TRV130 and PZM21 in CPP, however, and while these antinociceptive doses were ineffective in CPP, the lack of testing over multiple doses left the question of relative potency for antinociception and abuse-related effects unanswered. The current data demonstrate that TRV130 is equi-potent and equi-efficacious in reinforcing the effect of oxycodone, one of the most commonly-abused prescription opioids (Warner et al., 2016). Furthermore, TRV130 was equipotent to oxycodone at producing thermal antinociception, suggesting that therapeutic doses of TRV130 will at least be proximal to those that produce abuse-related effects.

It should be noted that the current study was not designed test an antnociceptive dose of TRV130 in self-administration directly. Rather, the aim was to conduct thorough dose-response characterizations of TRV130 and oxycodone in abuse- and therapeutic-related endpoints to determine if the relative potencies of TRV130 and oxycodone varied as a function of the endpoint. Given that the potencies of TRV130 and oxycodone are similar in self-administration and thermal antinociception, we see no evidence that biasing a mu-agonist towards G-protein signaling will improve the therapeutic index described above. However, we feel it is important to note that there are a number of other mu-agonist side effects that are reduced with TRV130 (see Singla et al. 2017; Soergal et al., 2014a; Soergal et al., 2014b; Viscusi et al. 2016), and that retaining abuse liability should not overshadow the significant progress in analgesic development that biased mu- agonists represent.

When the current results are taken together with previous findings, a plausible next step will be to test mu-agonists that exceed TRV130 in bias magnitude for G-protein signaling. As newer biased mu-agonists are developed, it will become possible to systematically test the degree of bias factor (i.e., degree of difference between potencies for G-protein signaling and signaling for other pathways) as a determinant of therapeutic/abuse-related effect ratios. However, doing so will require rigorous approaches that thoroughly characterize abuse-related and antinociceptive effects of biased mu-agonists in terms of potency and efficacy. It will also be important to test other modalities of pain, as potency for antinociception for

many drugs is known to vary across different modalities. It will also be of interest to test biased mu-agonists for tolerance-producing effects under self-administration baselines.

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Highlights

- Nicotine pre-treatment attenuated the avoidance induced by commonly abused drugs
- Nicotine had no impact on avoidance induced by lithium chloride
- Female rats appear to be more sensitive to these effects of nicotine
- Nicotine reduced sensitivity to the CTR stimulus properties of these drugs

Figure 1. Self-administration of oxycodone and TRV130 under a progressive-ratio schedule of reinforcement in male rats (n=7). Each data point represents the mean infusions earned at a dose for oxycodone and TRV130 (0.01–0.32 mg/kg/inj). TD = Training dose (0.056 mg/kg oxycodone). Sal = Saline. Filled symbols indicate a significant difference from saline. Error bars indicate $\pm$ SEM.

Figure 2. Antinociceptive effects of oxycodone (0.1–5.6 mg/kg, s.c.) and TRV130 (0.1–3.2 mg/kg, s.c.) on a Hot-Plate apparatus in rats (n=7). MPE = Maximum Possible Effect. Error bars indicate $\pm$ SEM.

Table 1.

Potency of oxycodone and TRV130 in self-administration and thermal antinociception (Hot-Plate test). ED₅₀ values for aggregated dose-response data for oxycodone and TRV130 are expressed in terms of mg/kg body weight.

	Oxycodone ED ₅₀ (95% C.I.)	TRV130 ED ₅₀ (95% C.I.)	Oxycodone ED ₅₀ / TRV130 ED ₅₀
Self-administration	0.039 (0.025 – 0.059)	0.026 (0.017 – 0.039)	1.50
Thermal antinociception	1.7 (1.06 – 2.56)	1.25 (0.79 –1.99)	1.36