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Tramadol acts as a weak reinforcer in the rat self-administration model, consistent with its low abuse liability in humans

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ABSTRACT

Rodent models of abuse potential are considered to represent a false positive with respect to the low risk of abuse liability associated with the atypical opioid analgesic tramadol. This may reflect either the predictive limitations of the models used to formulate this proposition (drug discrimination and conditioned place preference) or the predictive ability of the rodent per se. To address this concern, we used the rat self-administration model to examine the reinforcing properties of tramadol (0.3–3 mg/kg/infusion) under fixed (FR) and progressive-ratio (PR) schedules of reinforcement. Comparisons were made with the typical opioid analgesics morphine (0.03–0.3 mg/kg/infusion) and remifentanil (0.001–0.03 mg/kg/infusion). All three compounds maintained responding under an FR3 schedule of reinforcement, although clear differences were observed in the rates of responding between compounds. Under a PR schedule, morphine and remifentanil maintained comparable break points, while break points for tramadol did not differ from vehicle. Thus, when examined in the self-administration model, tramadol acts as a relatively weak reinforcer in rodents. These data are consistent with the low risk of tramadol abuse liability in humans and highlight the value of using multiple abuse potential models for assessing abuse liability.

1. Introduction

Understanding the abuse liability of novel compounds is a key component in the drug development process and is essential from a public health perspective. Abuse liability can be defined as the repeated taking of a drug for its reinforcing or subjective effects, or the avoidance of associated negative effects. In addition, abuse liability can be influenced by many factors such as drug formulation, ease of synthesis, cost, availability and the social acceptability of drug taking (Katz and Goldberg, 1988). Predicting abuse liability therefore requires use of a broad range of both clinical and non-clinical models that assess each of the distinct factors that can contribute to risk of abuse (that is, models of abuse potential).

Non-clinical animal models of abuse potential offer a particulary valuable resource for predicting abuse liability. These models include, but are not limited to, self-administration (Balster and Bigelow, 2003; Johanson, 1990; Panlilio and Goldberg, 2007), drug discrimination (Balster, 1991; Colpaert, 1999; Solinas et al., 2006), physical dependence and withdrawal (Emmett-Oglesby et al., 1990) and conditioned place preference (CPP; Tzschentke, 2007). The interpretation and design of these abuse potential models is critical for providing a thorough assessment of abuse liability, since each model provides insight into distinct behavioral effects of a compound and study outcomes can be influenced by a variety of experimental variables (Ator and Griffiths, 2003; Carter and Griffiths, 2009; Sanchis-Segura and Spanagel, 2006).

Abuse potential studies of the atypical opioid analgesic (±)-trans-2-(dimethylaminomethyl)-1-(m-methoxyphenyl)-cyclohexanol hydrochloride (tramadol) are potentially relevant in this context. Tramadol is considered as an atypical opioid due to its μ-opioid receptor and monoaminergic activity (see Grond and Sablotzki, 2004 for review). Clinical reports have indicated that the incidence of tramadol abuse liability is far lower than typical opioid analgesics (see Epstein et al., 2006 for review) and tramadol remains an uncontrolled substance under the Controlled Substances Act of 1970. In rhesus monkeys trained to self-administer the atypical opioid lefetamine, tramadol was reported to produce reinforcing effects that were considerably less than either the mixed opioid receptor agonist-antagonist pentazocine or the μ-opioid receptor (MOR) agonist morphine; drugs with high abuse liability (Yanagita, 1978). Thus, self-administration studies in non-human primates correlate well with clinical reports of abuse liability. In rodents, tramadol induces place preference (Sprague et al., 2002; Tzschentke et al., 2002) and generalizes to the discriminative stimulus produced by morphine in the drug discrimination model (Ren and Zheng, 2000). Based on these reports, it has been suggested that models of abuse potential in rodents represent a false positive with respect to the observed abuse liability of tramadol (Epstein et al., 2006). Epstein et al. (2006) concluded that rodent studies were useful but not sufficient for predicting abuse liability and that species differences in metabolic

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pathways and receptor profiles, for example, may result in misleading conclusions regarding the abuse liability of some compounds.

An alternative explanation for the overestimation of tramadol abuse liability, based on rodent drug discrimination and CPP models, is that these models were unable to evaluate effects of tramadol on certain drug-induced or maintained behaviors that likely contribute to an increased risk of abuse liability. Specifically, the drug discrimination model can identify the interoceptive cue (often equated to the subjective sate) induced by a drug, and CPP can assess the conditioned reinforcing properties of environmental stimuli associated with drug experience, or may reflect other psychologically distinct learned associations between the drug and the context in which it was received (Stephens et al., 2010). However, neither model can be used to directly assess the primary reinforcing effects of a drug (that is, the ability of a drug to increase the subsequent probability of a response that precedes its delivery), nor the relative reinforcing efficacy of a drug (that is, how reinforcing is a drug in comparison to other drugs). Understanding the primary and relative reinforcing effects of drugs are critical components for predicting abuse liability.

The self-administration model, in which animals are required to elicit a response (such as a lever press or nose-poke into an aperture) in order to obtain drug is considered as the gold-standard model for assessing primary reinforcement (Carter and Griffiths, 2009). Self-administration procedures that examine relative reinforcing efficacy have also been described (see Katz, 1990 for review), of which the progressive-ratio (PR) schedule is perhaps the most widely used (Richardson and Roberts, 1996). In a PR schedule, the response demand requirement to obtain drug is progressively increased until a point at which the animal no longer responds. This 'break point' can be used to compare the relative reinforcing efficacy of different drugs, or different doses of the same drug. To this extent, the present study used the self-administration model to examine both the primary and relative reinforcing properties of tramadol in the rat, with comparisons made to the μ-opioid receptor agonists remifentanil and morphine. Our findings show that tramadol serves as a weak reinforcer in the rat, relative to drugs with known abuse liability, which is consistent with findings reported in non-human primates (Yanagita, 1978) and the lack of widespread abuse of tramadol in humans (Epstein et al., 2006).

2. Materials and methods

2.1. Subjects

Subjects were sixteen experimentally naïve male Hooded Lister rats (Charles River Laboratories, Kent, UK). Rats were housed in polycarbonate cages in groups of 4–6 per cage on arrival in the vivarium and allowed to acclimatize to the holding room for a minimum of five days prior to use. Rats weighed approximately 250–300 g at the time of surgery and were single housed following the surgical procedure to prevent damage to the implanted jugular vein catheter (JVC). All subjects had free access to water except during the self-administration tests. Rats were fed approximately 20 g of standard lab chow (Rat and Mouse No.1 Maintenance diet; Special Diet Services, Essex, UK) per day. Daily feeding occurred at approximately 2 h after the experimental session or at approximately 11:00 h if no experiments were performed on that day. Rats were maintained under a 12:12 h light/dark cycle (lights on 07:00 h) with temperature and humidity controlled at 21 ± 2 °C and $55 \pm 10\%$, respectively, in both the holding and test room. All experiments were performed in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act.

2.2. Drugs

Remifentanil hydrochloride (Ultiva; GlaxoSmithKline, Middlesex, UK) was supplied in vials containing 1 mg remifentanil and 15 mg glycine in dry powder form. Each vial was mixed with 1 ml 0.9% sterile saline to produce a 1 mg/ml remifentanil solution with 15 mg/ml glycine. This solution was further diluted in 0.9% sterile saline to give concentrations of remifentanil at 0.015–0.45 mg/ml. The vehicle for remifentanil was 0.9% saline containing 0.75 mg/ml glycine (Sigma-Aldrich, Dorset, UK), which was an equivalent concentration of glycine to that in the highest remifentanil concentration. Morphine hydrochloride (Macfarlan Smith, Edinburgh, UK) and tramadol hydrochloride (Tocris Bioscience, Bristol, UK) were dissolved in 0.9% sterile saline to give concentrations of 0.6–6 mg/ml and 5–50 mg/ml, respectively. All drug concentrations and doses refer to the active moiety.

2.3. Surgery

One day prior to surgery, rats were provided with antibiotic (Baytril; 100 mg/l of a 2.5% oral solution: Bayer Animal Health Division, Newbury, UK) in the drinking water. On the day of surgery, anti-inflammatory treatment and analgesia was provided (Rimadyl; 5 mg/kg s.c.: Pfizer Ltd, Sandwich, UK). Rats were anaesthetized with isoflurane (Isoflo; Abbott Laboratories, Queenborough, UK)/oxygen and a jugular vein catheter (model IVSAp40; CamCaths, Cambridge, UK), previously sterilized by exposure to ethylene oxide gas, was implanted. The proximal end was placed at the right atrium, entering at the right jugular vein, while the distal end was passed over the right shoulder and exited dorsally, between the scapulae. Rimadyl and Baytril treatment continued for three and five days post-surgery, respectively. A minimum of seven days post-surgery recovery was allowed prior to use on experiments. Catheter patency was maintained with daily i.v. infusions of 0.1 ml heparinised (50 units/ml) sterile 0.9% saline. When rats were not used for periods longer than 48 h, catheters were locked with heparinised (50 units/ml) glycerol. Cather patency was assessed by injection of 0.1 ml propofol (Diprivan; AstraZeneca, Cheshire, UK).

2.4. Apparatus

Self-administration training and testing occurred in operant chambers (ENV-008CT; Med-Associates, Vermont, USA) individually housed within sound attenuating cubicles, which were ventilated by an exhaust fan that also served to mask external noise. On one wall of the operant chamber were located two retractable response levers (ENV-112CM; Med-Associates), located 12 cm apart and 6 cm from the grid floor. A single cue light (ENV-221M; Med-Associates) was located approximately 4 cm above each lever and a house light (ENV-215 M; Med-Associates) was located on the wall opposite to the response levers. External to the sound attenuating cubicle was located a fixed-rate syringe pump (PHM100; Med-Associates). Drug was infused via a length of plastic tubing (PE50; Plastics One, Virginia, USA) connected from the infusion syringe to a stainless steel single channel swivel (Model 375/22; Instech Laboratories, Pennsylvania, USA) mounted directly above the operant chamber on a counter-balanced lever arm (PHM-110; Med-Associates). A further length of tubing, shielded by a metal spring tether, connected from the swivel to the external guide cannulae of the implanted JVC. Operant chambers were controlled and data recorded, using Med-PC IV software (Med-Associates).

2.5. Procedure

Rats were placed into the operant chambers and self-administration sessions commenced with an automatic infusion designed to fill the JVC with drug. The house light and the cue light located above the active lever were then illuminated and both response levers were extended into the operant chamber. Responding on one

lever (the active lever) resulted in drug delivery, followed by a timeout period of 5 s, during which time the cue light located above the active lever was extinguished and further responding on the active lever had no scheduled consequence. Responding on the alternative lever (the inactive lever) had no scheduled outcome throughout the experimental session. Active and inactive levers were randomly assigned to each rat. The unit dose of drug available was determined by the unit volume per infusion adjusted for the weight of the rat, which was controlled by changing the duration of activation of the infusion pump. The duration of infusion was maintained at between 1 and 3 s by altering the stock concentration of drug. All experiments were performed between 08:00 and 18:00 h, up to five days per week. FR sessions were 1 h in duration and PR sessions were 4 h in duration.

2.5.1. Remifentanil self-administration training

All rats were initially trained to respond for remifentanil (3 μg/kg/infusion) under a fixed-ratio 1 (FR1) schedule of reinforcement (that is, one press on the active lever resulted in a single infusion of remifentanil) during 1 h sessions. Once ≥7 infusions of remifentanil had been obtained in a single session, the response requirement was increased from an FR1 to an FR2 schedule, and subsequently to an FR3 schedule of reinforcement. When rats were obtaining >16 infusions per session, and were demonstrating stable self-administration of remifentanil under an FR3 schedule (as defined by ≤20% variation in the number of infusions obtained during two consecutive sessions), responding was extinguished by replacing remifentanil infusions with vehicle. Subjects then progressed to test sessions when a stable baseline level of responding for vehicle had been established, as defined by i. the mean number of vehicle infusions between two consecutive sessions being ≤50% the mean number of infusions obtained during the final two sessions of stable responding for remifentanil and ii. ≤20% variation in the number of vehicle infusions obtained between two consecutive sessions or iii. within ±4 vehicle infusions from one session to the next, where the number of infusions obtained during one session was ≤10.

2.5.2. Self-administration of remifentanil, tramadol and morphine under a fixed-ratio schedule of reinforcement

Unit doses of remifentanil (0, 1, 3, 10 and 30 μg/kg/infusion), tramadol (0, 0.3, 1 and 3 mg/kg/infusion) and morphine (0, 0.03, 0.1 and 0.3 mg/kg/infusion) were tested in three groups of rats under an FR3 schedule of reinforcement. All dose-effect functions were determined using a within-subject design with doses presented in a counterbalanced order. Each dose was tested until stable responding was observed over two consecutive test sessions. In addition to recording lever response rates and the number of drug infusions obtained in each 1 h session, the mean post-infusion pause (defined as the time from each infusion to the next lever response) was calculated to provide an additional measure of the behavioral effects of the available drug doses. On completion of a dose-effect function, responding for the training dose of remifentanil was re-established, or catheter patency tested with propofol prior to testing of further drugs. Once dose–response determinations had been completed, rats were moved onto progressive-ratio tests.

2.5.3. Self-administration of remifentanil, tramadol and morphine under a progressive-ratio schedule of reinforcement

Doses of remifentanil (0, 1, 3, 10 and 30 μg/kg/infusion), tramadol (0, 0.3, 1 and 3 mg/kg/infusion) and morphine (0, 0.03, 0.1 and 0.3 mg/kg/infusion) were tested in three groups of rats (where catheter patency permitted, some rats were used for testing two of the three compounds, resulting in final *n*'s per drug of 7, 7 and 7, respectively) under a PR schedule of reinforcement in which the FR requirement increased following each drug infusion according to the following sequence: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603. The break point was recorded, which was defined as the number of infusions obtained prior to a 30 min period where no further infusions were obtained. All PR dose-effect functions were determined using a within-subject design, with doses presented in a counterbalanced order. Each dose was tested until a stable break point was observed between two consecutive test sessions.

2.6. Statistical analysis

The mean of data from the final two sessions under each condition was used for statistical analysis. Analysis of the acquisition of remifentanil self-administration was performed using paired-samples *t*-tests to compare response rates for remifentanil to response rates following extinction, and to compare response rates on the active and inactive levers for remifentanil self-administration. For FR dose-effect determinations, dependent variables used were infusion rates, response rates on both active and inactive levers and post-infusion pauses. Response rates were analyzed using a two-way, within-subjects Analysis of Variance (ANOVA), with lever and test-drug dose as factors. Infusion rates and post-infusion pause data were analyzed using a one-way, within-subjects ANOVA with the factor of test drug-dose. For PR dose-effect determinations the dependent variable was the break point. Break points were analyzed using a one-way, within-subjects ANOVA with the factor of drug dose. Post-hoc comparisons were performed to compare each dose of test drug to the vehicle of that drug using Dunnett's test. Statistical analysis was performed using Statistica v7.1 (StatSoft Inc, Oklahoma, USA).

3. Results

3.1. Acquisition of remifentanil self-administration

In agreement with a previous report (Panlilio and Schindler, 2000), rats successfully acquired self-administration of remifentanil as indicated by a significant increase in active lever response rates compared to inactive lever response rates over the last three sessions ($df=15$, $t=3.82$, 5.73 and 4.74 respectively, $p<0.01$). When remifentanil was replaced with vehicle, extinction of responding was confirmed by comparison of active response rates for remifentanil with active response rates for vehicle ($df=15$, $t=4.40$, $p<0.01$). Analysis of response rates indicated that selective responding on the active lever was retained during extinction, though clearly at a level lower than that observed during remifentanil self-administration ($df=15$, $t=7.36$, 3.67 and 4.66 respectively, p's<0.01; Fig. 1).

3.2. Self-administration of remifentanil, tramadol and morphine under a fixed-ratio schedule of reinforcement

When rats were given the opportunity to self-administer remifentanil, infusion rates varied with dose (Fig. 3A) and ANOVA confirmed a significant effect of dose on infusion rates ($F(4,24)=28.35$, $p<0.01$). Post-hoc comparisons revealed a significant increase in infusion rates at the 0.001 and 0.003 mg/kg/infusion doses in comparison to vehicle (p's<0.01). Responding for remifentanil was selective for the active lever and varied with dose (Fig. 2A), as indicated by a lever by dose interaction ($F(4,24)=23.05$, $p<0.01$). Post-hoc comparisons indicated a significant increase in response rates on the active lever at 0.001 and 0.003 mg/kg/infusion (p's<0.01).

When rats were given the opportunity to self-administer morphine, infusion rates varied with dose (Fig. 3A) and ANOVA confirmed a significant effect of dose on infusion rate ($F(3,21)=7.63$, $p<0.01$) with post-hoc comparisons revealing a significant increase in infusion rates at the 0.1 mg/kg/infusion dose in comparison to vehicle

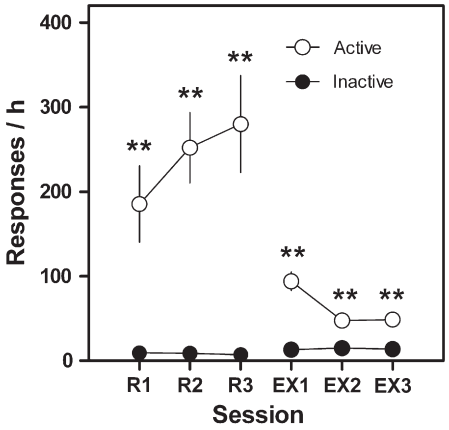

Fig. 1. Acquisition and extinction of responding for remifentanil. Data show mean (± SEM) response rates on the active and inactive levers during the final three 1 h training sessions for 0.003 mg/kg/infusion remifentanil (R1–R3), and the first three 1 h extinction (EX1–EX3) sessions maintained under a fixed-ratio 3 schedule of reinforcement. Although a preference for responding on the active lever was maintained during extinction sessions, active lever responding during extinction sessions was significantly reduced in comparison to responding during training sessions (comparison of mean active lever response rates from R2–R3 vs. EX2–EX3, $p<0.01$). $^{**}p<0.01$ compared to responding on the inactive lever for the session.

($p<0.01$). Responding for morphine was selective on the active lever and varied with dose (Fig. 2B), as indicated by a lever by dose interaction ($F(3,21)=3.59$, $p<0.05$). Post-hoc comparisons indicated a significant increase in response rates on the active lever at the 0.1 mg/kg/infusion dose ($p<0.01$).

When rats were given the opportunity to self-administer tramadol, infusion rates did not vary with dose (Fig. 3A) as confirmed by ANOVA ($F(3,21)=2.14$, not significant (n.s.)). Analysis of response rates for tramadol (Fig. 2C) revealed significant effects of lever ($F(1,7)=30.64$, $p<0.01$) and dose ($F(3,21)=3.81$, $p<0.05$). Post-hoc comparisons indicated a significant increase in response rates on the active lever at the 1 mg/kg/infusion dose ($p<0.05$). No significant effect of dose was observed for inactive lever responding ($F(3,21)=1.79$, n.s.).

Analysis of the post-infusion pause indicated that both remifentanil and tramadol increased the duration of this variable (Fig. 3B). For remifentanil self-administration, analysis of the post-infusion pause indicated a significant main-effect of dose ($F(4,24)=8.84$, $p<0.01$) and *post-hoc* comparisons indicated a significant increase in the post-infusion pause at the 0.03 mg/kg/infusion unit dose ($p<0.01$). During tramadol self-administration, the post-infusion pause increased as a function of dose ($F(3,21)=5.39$, $p<0.01$) and post-hoc comparisons indicated a significant increase in the post-infusion pause at the 3 mg/kg/infusion unit dose ($p<0.01$). During morphine self-administration, no changes compared to vehicle were observed in the post-infusion pause ($F(3,21)=1.29$, n.s.). During vehicle self-administration sessions, rats typically responded rapidly at the beginning of each session, but then made very few (if any) responses during the remainder of the session. This response profile was reflected by a relatively short post infusion pause value (Fig. 3B).

3.3. Self-administration of remifentanil, tramadol and morphine under a progressive-ratio schedule of reinforcement

Both morphine and remifentanil maintained responding at levels which exceeded that of the respective vehicles (Fig. 4). For morphine self-administration, analysis of break point data with ANOVA revealed a significant effect of dose ($F(3,18)=7.78$, $p<0.01$), with post-hoc tests confirming a significant increase in break point compared to vehicle, at the 0.1 and 0.3 mg/kg/infusion

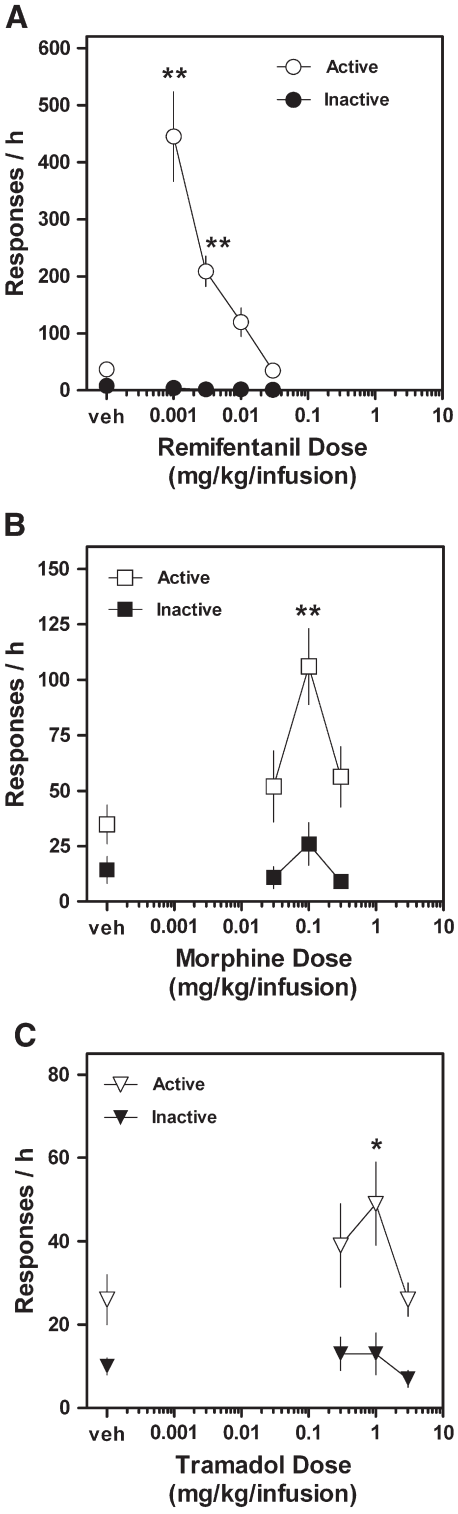

Fig. 2. Self-administration of A) remifentanil, B) morphine and C) tramadol under a fixed-ratio 3 schedule of reinforcement. Data show mean (± SEM) response rates on the active and inactive levers during 1 h self-administration sessions. Each data point represents average response rates during the final two sessions of access to each dose. $^{**}p<0.01$, $^{*}p<0.05$ compared to responding for vehicle on the active lever. Note that different scales are used for the *y*-axis of each panel.

doses ($p<0.05$ and $p<0.01$ respectively). For remifentanil self-administration, analysis of break point data with ANOVA revealed a significant effect of dose ($F(4,24)=5.23$, $p<0.01$), and post-hoc tests indicated a significant increase in break point in comparison to vehicle at the 0.03 mg/kg/infusion dose ($p<0.01$). No increase in

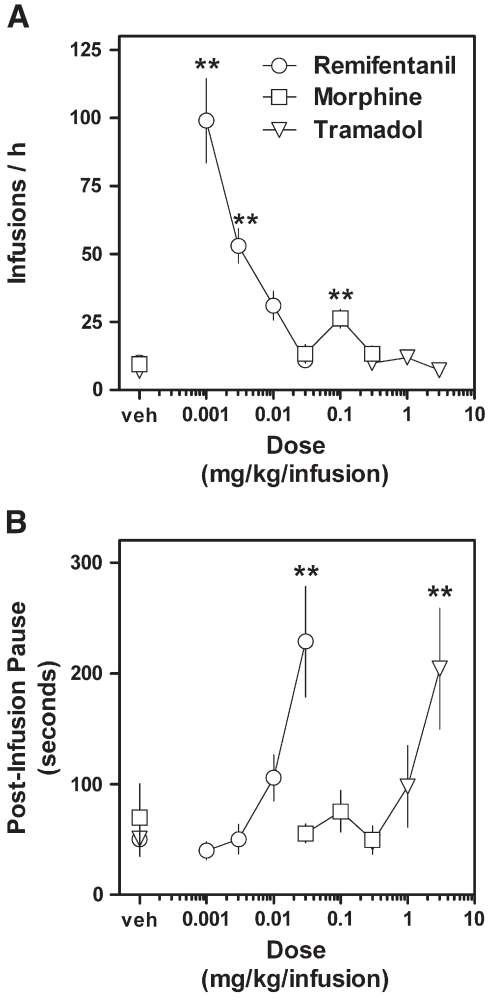

Fig. 3. Infusion rates and post-infusion pauses maintained by remifentanil, morphine and tramadol under a fixed-ratio 3 schedule of reinforcement. A) Data show mean (± SEM) infusion rates for remifentanil, morphine or tramadol during 1 h self-administration sessions. B) Data show the mean (± SEM) post-infusion pause that occurred following infusions of remifentanil, morphine or tramadol during 1 h self-administration sessions. The post-infusion pause was defined as the time from each infusion to the next lever response. Each data point represents averages during the final two sessions of access to each dose. $^{**}p<0.01$, $^{*}p<0.05$ compared to respective vehicle.

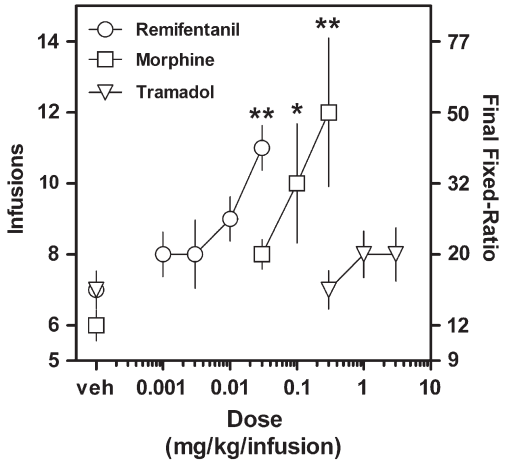

Fig. 4. Self-administration of remifentanil, morphine and tramadol under a progressive-ratio schedule (PR) of reinforcement. Data show mean (± SEM) break points (expressed as number of infusions earned and final fixed-ratio (FR) completed) for remifentanil, morphine or tramadol during 4 h self-administration sessions. The break point was defined as the number of infusions obtained prior to a 30 min period where no further infusions were obtained. Each data point represents averages during the final two sessions of access to each dose. $^{**}p<0.01$, $^{*}p<0.05$ compared to respective vehicle.

break points compared to vehicle were observed for any unit dose of tramadol ($F(3,18)=0.70$, n.s.; Fig. 4).

4. Discussion

The present study investigated the primary and relative reinforcing effects of tramadol in rats, using the self-administration model, to determine whether the purported overestimation of tramadol abuse liability based on rodent studies of abuse potential (Epstein et al., 2006) reflected the predictive limitations of the models used for this proposition (drug discrimination and CPP), or the predictive ability of the rodent per se. We report that tramadol can serve as a primary reinforcer in rodents when assessed under an FR schedule of reinforcement. However, our study shows that the relative reinforcing effects of tramadol, assessed under a PR schedule of reinforcement, are weak in comparison to typical opioid analgesics with recognized abuse liability (morphine and remifentanil). Our findings are consistent with those reported using the self-administration model in non-human primates (Yanagita, 1978) and accurately reflect the low risk of tramadol abuse liability observed in humans (Epstein et al., 2006). Critically, our report highlights that predictions of abuse liability, when made from data obtained in any one species, are greatly strengthened when multiple aspects of drug effects on behavior are taken into account.

We report that tramadol can serve as a primary reinforcer, as indicated by a significant increase in active lever responding at the 1 mg/kg/infusion dose under an FR3 schedule of reinforcement, although tramadol infusion rates maintained under this schedule failed to significantly differ from vehicle at any dose tested. The dissociation between response and infusion rates was due to a small increase in active lever responses during the time-out period following each drug infusion. In marked contrast, both response rates and infusions rates were significantly higher for remifentanil and morphine. It is interesting to note that our findings are remarkably consistent with those reported in rhesus monkeys by Yanagita (1978), showing that tramadol produced an increase in infusion rates, but this effect failed to reach statistical significance.

Response rates maintained under FR schedules of reinforcement not only reflect primary reinforcement, but can also be influenced by satiety, adverse effects and the pharmacokinetic properties of a drug, with short-acting compounds maintaining higher rates of responding (Ator and Griffiths, 2003; Lau and Sun, 2002; Norman and Tsibulsky, 2006; Tsibulsky and Norman, 1999). Thus, the finding that remifentanil maintained higher response rates than either morphine or tramadol reflects remifentanil's extremely short duration of action (Michelsen and Hug, 1996), which presumably produces only a brief period of satiety after each infusion.

It follows that a potential explanation for the observed low rates of responding for tramadol under FR or PR schedules may have been due to inappropriate dose selection, since the dose range used maintained responding that only slightly differed from vehicle when examined using a FR schedule. This account is unlikely since the doses chosen were behaviorally active, as demonstrated by an increase in the post-infusion pause following each infusion of tramadol at the higher unit doses. Post-infusion pauses may reflect sedation and/or satiation of reinforcement. While it is possible that the sedative properties of a compound may inhibit the ability of rats to continue self-administration behavior, this does not appear to be the case with tramadol. The magnitude of the increase in the post-infusion pause observed with tramadol was similar to that observed with remifentanil, which still maintained remarkably high rates of self-administration.

One of the key challenges for non-clinical (and indeed clinical) abuse potential assessments is to understand the relative reinforcing efficacy of drugs. This is a broader topic than the discussion here

permits, and one which has been extensively reviewed by others (e.g. Katz, 1990; Stafford et al., 1998). The PR schedule, as applied for our study, is considered as a useful and applicable measure of relative reinforcing strength (Brady and Griffiths, 1976). Further, non-clinical assessments of relative reinforcing efficacy using PR schedules have been reported to translate well to clinical reports of both subjective effects and the dependence producing capacity of a variety of abused opioids (Hoffmeister, 1979). Our report confirms that the PR schedule provides an accurate measure of relative reinforcing efficacy when used to compare atypical and typical opioids. Alternative PR schedules have been proposed for assessing opioids, which vary in the rate at which the demand requirement for obtaining drug increases either within (Grasing et al., 2003) or between (Roberts and Bennett, 1993) self-administration sessions. Such schedules may prove beneficial for minimizing the probability that sensitization or tolerance to the reinforcing effects of the test drug may develop (see Arnold and Roberts, 1997 for further discussion). It is unlikely that sensitization or tolerance could have contributed to the effects reported in our study, since we used a within-subjects design in which the order of doses tested were counterbalanced within each drug group.

Non-clinical assessments of abuse potential form an integral part of the drug development process in providing an early understanding of the likelihood of abuse liability for novel compounds. But, a challenge for researchers is to decide which abuse potential models to employ. In addition to studies of pharmacology, which can be used to identify pharmacological actions associated with an increased risk of abuse liability, a variety of animal models of abuse potential are on offer, each of which can measure different aspects of drug-induced or maintained behavior that can contribute to abuse liability (see Ator and Griffiths, 2003; Sanchis-Segura and Spanagel, 2006; Tzschentke, 1998 for review). For the purpose of predicting abuse liability, non-clinical assessments that incorporate data from studies of pharmacology with findings from a range of in vivo abuse potential models are favored from a scientific and regulatory perspective (EMEA, 2006). As the following discussion demonstrates, our current findings with tramadol clearly support this proposition.

From studies of pharmacology, tramadol is identified as a racemate consisting of two enantiomers that display different binding and pharmacological profiles. (+)-Tramadol and the major metabolite (+)-O-desmethyl-tramadol (M1) are MOR agonists, although M1 has at least 400 fold greater affinity than (+)-tramadol at MOR. (+)-Tramadol also acts as a serotonin reuptake inhibitor, while (−)-tramadol and the (−)-M1 metabolite inhibit noradrenaline reuptake (see Gibson, 1996 and; Grond and Sablotzki, 2004 for review). This diverse pharmacological profile of tramadol likely contributes to findings from non-clinical (and indeed clinical) studies of abuse potential and the low abuse liability of tramadol in humans. First, the weak reinforcing effects of tramadol reported in our present study may be attributable to the serotonergic and noradrenergic actions of tramadol inhibiting reinforcement that could otherwise be mediated by tramadols MOR agonist effects. This proposal is supported by studies showing that both dexfenfluramine (a selective serotonin releaser/reuptake inhibitor) and venlafaxine (a noradrenaline reuptake inhibitor) partially reduce self-administration of heroin (a MOR agonist) in rats (Magalas et al., 2005; Wang et al., 1995). Second, the reinforcing effects of tramadol may be further limited due to the delay in onset of tramadols MOR-agonist effects, which are mainly mediated by its (+)-M1 metabolite, which is formed in the liver via CYP2D6 (Grond and Sablotzki, 2004). The necessity for rapid rates of receptor occupancy in maintaining reinforcement in animals is well accepted (for example, Woolverton and Wang, 2004), and a clinical study has highlighted the requirement for rapid intravenous delivery of morphine to engender reinforcement-like effects (that is, reports of positive subjective effects) in humans (Marsch et al., 2001). Third, the route of tramadol administration used in the present study, and which is commonly used by drug abusers seeking to achieve a drug high or rush (that is, intravenous), may actually reduce rather than increase the MOR-agonist effects of tramadol. Analysis of tramadols pharmacokinetic profile in rats has revealed that oral or intraperitoneal (i.p.) delivery of tramadol is associated with stereoselective metabolism of tramadol in favor of (+)-M1, while intravenous delivery of tramadol does not result in stereoselective pharmacokinetics (Parasrampuria et al., 2007). Moreover, the influence of differences in the biotransformation of tramadol, as a function of administration route, on findings from abuse potential studies is highlighted by reports from Epstein et al. (2006), who show that tramadol engenders reinforcement-like effects when administered orally in humans, but not intravenously. Clearly then, predictions of abuse liability from non-clinical (and clinical) studies of abuse potential are greatly strengthened when consideration is given to the pharmacological and metabolic profile of the test drug.

In the drug discrimination model, used to identify the interoceptive state (akin to the subjective effect) induced by a drug, tramadol was shown to produce full generalization to morphine in Sprague–Dawley rats, an effect that was blocked by the opioid receptor antagonist naloxone (Ren and Zheng, 2000). Together with other reports (Filip et al., 2004; Swedberg et al., 1992, 1988), rodent drug discrimination studies indicate that the interoceptive state of tramadol is predominantly mediated through opioid mechanisms. While the identification of interoceptive states can provide valuable information regarding the in vivo pharmacology of a drug, these states can occur independently of drug reinforcement (Lamb et al., 1991), which is a critical consideration for understanding abuse liability.

The CPP model can be used to assess conditioned drug reinforcement (Tzschentke, 1998). Tramadol was reported to produce CPP in Sprague–Dawley rats comparable to that induced by morphine, and tramadol CPP was blocked by pre-treatment with naloxone (Sprague et al., 2002; Tzschentke et al., 2002). Interestingly, Tzschentke et al. (2002) noted that while morphine induced behavioral sensitization during CPP training, tramadol did not. Based on the proposed role of sensitization in addictive processes (Robinson and Berridge, 1993), this finding was interpreted as being consistent with the lack of significant tramadol abuse liability. However, care must be exercised when using the CPP model to understand abuse liability. First, there is considerable debate concerning the psychological processes that contribute to the development of CPP, with suggestions that place preference could reflect conditioned reinforcement, conditioned approach and/or discriminated approach responses (Mead et al., 2005; Sanchis-Segura and Spanagel, 2006; Stephens et al., 2010). These psychologically distinct learning processes are important to consider since they are underpinned by different neural substrates (Everitt et al., 1999; Mead and Stephens, 2003a,b; O'Connor et al., 2010) and are therefore likely to respond differently to drug challenges. Second, a number of drugs that are devoid of abuse liability can produce place preference (e.g. clonidine, antidepressents and NSAIDs; Tzschentke, 1998). Thus, the CPP model, while providing a valuable insight to learning processes proposed to contribute to addiction, can lack the selectivity required to measure the primary and relative reinforcing effects of drugs necessary for understanding abuse liability.

Our current study employed the self-administration model to examine the primary reinforcing effects of tramadol in the rat and compare its reinforcing effects to those of two typical opioid agonists with known abuse liability. Findings from our study accurately reflect the low risk of tramadol abuse liability observed in humans (Epstein et al., 2006), which adds further support to previous reviews reporting high predictive validity of the self-administration model for abuse liability (Johanson and Balster, 1978). However, akin to other in vivo abuse potential models, self-administration study outcomes can be influenced by a number of experimental variables (Ator and Griffiths, 2003), and findings of drug reinforcement in animals are not always associated with abuse liability in humans (for example, bupropion

and modafinil; Bergman et al., 1989; Griffith et al., 1983; Myrick et al., 2004; Tella et al., 1997). For these reasons, we argue that non-clinical self-administration data is of greatest value for understanding the risk of abuse liability for novel compounds when used in conjunction with data from studies of pharmacology and findings from other in vivo abuse potential models, as exemplified in the above discussion with respect to tramadol.

In summary, the data reported here are consistent with the clinical profile of abuse liability associated with tramadol. Tramadol is not devoid of reinforcing effects, but these effects are weak in comparison to the typical μ-opioid receptor-agonists remifentanil and morphine. By using a variety of in vivo abuse potential models and incorporating data generated in these models with other facets of drug effects, such as in vitro binding, pharmacokinetic–pharmacodynamic and adverse event profiles, through to clinical pharmacology and post marketing surveillance, one can begin to build a picture relating to the likelihood of abuse relative to other drugs with known abuse liability. As commented by Katz and Goldberg (1988), since the abuse of drugs in humans is a function of societal variables in addition to pharmacological factors, it is unlikely that non-clinical abuse potential studies will ever yield more than qualitative information on abuse liability. Nevertheless, our report highlights that using a progressive ratio schedule in the self-administration model can offer a valuable assessment of relative reinforcing efficacy, and that predictions of abuse liability can be greatly strengthened when a variety of abuse potential models, that measure different aspects of drug effects on behavior, are employed.

Conflict of interest

At the time the study was conducted, Eoin O'Connor was a Pfizer employee. Eoin O'Connor currently receives a DPhil studentship from the BBSRC and Pfizer Inc. Andy Mead is an employee of Pfizer Inc. This study was sponsored by Pfizer Inc.

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