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Role of mu-opioid agonist efficacy on antinociceptive interactions between mu agonists and the nociceptin opioid peptide agonist Ro 64-6198 in rhesus monkeys

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Abstract

Mu-opioid receptor agonists are clinically effective analgesics, but also produce undesirable effects that limit their clinical utility. The nociceptin opioid peptide (NOP) receptor system also modulates nociception, and NOP agonists might be useful adjuncts to enhance the analgesic effects or attenuate the undesirable effects of mu-opioid agonists. The present study determined behavioral interactions between the NOP agonist (–)-Ro 64-6198 and mu-opioid ligands that vary in mu-opioid receptor efficacy (17-cyclopropylmethyl-3,14β-dihydroxy-4,5α-epoxy-6α-[(3′-isoquinolyl)acetamido]morphinan (NAQ) < buprenorphine < nalbuphine < morphine = oxycodone < methadone) in male rhesus monkeys. For comparison, Ro 64-6198 interactions were also examined with the kappa-opioid receptor agonist nalfurafine. Each opioid ligand was examined alone and following fixed-dose Ro 64-6198 pretreatments in assays of thermal nociception (n=3-4) and schedule-controlled responding (n=3). Ro 64-6198 alone failed to produce significant antinociception up to doses (0.32 mg/kg, IM) that significantly decreased rates of responding. All opioid ligands, except NAQ and nalfurafine, produced dose- and thermal intensity-dependent antinociception. Ro 64-6198 enhanced the antinociceptive potency of buprenorphine, nalbuphine, methadone, and nalfurafine. Ro 64-6198 enhancement of nalbuphine antinociception was NOP antagonist SB-612111 reversible and occurred under a narrow range of dose and time conditions. All opioid ligands, except NAQ and buprenorphine, produced dose-dependent decreases in rates of responding. Ro 64-6198 did not significantly alter mu-opioid ligand rate-decreasing effects. Although these results suggest that NOP agonists may selectively enhance the antinociceptive vs. rate-suppressant effects of some mu-opioid agonists, this small

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Conflicts of Interest

The authors report no potential or perceived conflicts of interest.

enhancement occurred under a narrow range of conditions dampening enthusiasm for NOP agonists as candidate “opioid-sparing” adjuncts.

Keywords

mu-opioid receptor; nociceptin opioid peptide receptor; kappa-opioid receptor; rhesus monkey; warm water tail-withdrawal; antinociception

1. INTRODUCTION

Pain management remains a significant public health issue in the United States (Ballantyne et al., 2017). Mu-opioid receptor agonists (e.g. oxycodone) are one of the most effective pharmacological tools available to clinicians for the treatment of pain (Dowell and Haegerich, 2016). However, the clinical utility of mu-opioid agonists is severely limited by a number of undesirable effects including respiratory depression and sedation. One approach to enhance the clinical utility of mu-opioid agonists may be to combine them with other compounds that act through different receptor mechanisms (Di Cesare Mannelli et al., 2015; Dietis et al., 2009; Gunther et al., 2017).

One potential receptor system that might function as a useful mu-opioid agonist adjunct is the nociceptin opioid peptide (NOP) system. Two lines of evidence support potential interactions between mu-opioid and NOP receptors. First, NOP receptors are colocalized with mu-opioid receptors in both spinal and brain regions involved in nociceptive signaling pathways (for review, see Toll et al. 2016). Second, preclinical studies have reported species difference in the antinociceptive effects of the selective high-efficacy NOP agonist Ro 64-6198 (Jenck et al., 2000). For example, systemic Ro 64-6198 produced antinociception in monkeys (Ko et al., 2009), but not rodents (Reiss et al., 2008). Further highlighting potential species differences, systemic combinations of Ro 64-6198 and a mu-opioid agonist produced additive antinociceptive effects in mice (Reiss et al., 2008), but synergistic antinociceptive effects in rhesus monkeys (Cremins et al., 2012). Overall, this literature supports further evaluation of NOP and mu-opioid agonist interactions.

Previous studies examining mu-opioid agonist antinociceptive interactions with other receptor systems suggest that one important determinant of these interactions may be MOR agonist efficacy (Banks et al., 2010b; Maguire and France, 2014; Negus et al., 2009). However, the degree to which mu-opioid agonist efficacy is a determinant of NOP agonist interactions is unknown. Therefore, the aim of the present study was to determine the role of mu-opioid ligand efficacy in antinociceptive interactions with the NOP agonist Ro 64-6198 in rhesus monkeys using previously described procedures (Banks et al., 2010b; Stevenson et al., 2003). Antinociceptive interactions between Ro 64-6198 and six mu-opioid ligands (17-cyclopropylmethyl- 3,14β-dihydroxy-4,5α-epoxy-6α-[(3'-isoquinolyl)acetamido]morphinan (NAQ), buprenorphine, nalbuphine, morphine, oxycodone, and methadone) that vary in agonist-stimulated GTPγS binding from lowest to highest (Selley et al., 1998; Thompson et al., 2004; Zaidi et al., 2013) and in their in vivo effectiveness to produce antinociception (Cornelissen et al., 2018a) were investigated. For comparison, Ro 64-6198 interactions were also investigated with the selective high efficacy

kappa-opioid receptor agonist nalfurafine. Nalfurafine is not a clinically-approved analgesic and fails to produce antinociception under conditions that dissociate antinociception from behavioral sedation (Endoh et al., 2001; Lazenka et al., 2018). Drug interactions were also examined in an assay of schedule-controlled responding in a different cohort of monkeys to assess behavioral selectivity to produce antinociception vs. rate suppression. If NOP agonists are to be considered as candidate mu-opioid agonist adjuncts, then we would hypothesize that Ro 64-6198 would robustly and selectively enhance the antinociceptive vs. rate-suppressant effects of mu-opioid agonists.

2. MATERIALS AND METHODS

2.1 Subjects

A total of seven middle-aged adult (10-18 years old) male rhesus macaques (*Macaca mulatta*) of either Indian or Chinese origin and weighing between 10-14 kg served as subjects. Four monkeys served as subjects in the assay of thermal nociception, and three monkeys served as subjects in the assay of schedule-controlled responding. These sample sizes have been sufficient to detect mu-opioid agonist interactions in previous publications (Banks et al., 2010a; Banks et al., 2010b; Maguire and France, 2014; Schwienteck et al., 2018; Stevenson et al., 2003). All monkeys had experimental histories of opioid, monoamine transporter ligand, and *N*-methyl *D*-aspartate antagonist exposure. Diet was comprised of laboratory monkey chow (#5049, Purina, Framingham, MA) and supplemented with fresh fruits, vegetables, and nuts. All subjects were housed individually and had *ad lib* water access while in the housing chamber. A 12h light/dark cycle (lights on from 6:00 AM to 6:00 PM) was in effect. Housing facilities were licensed by the United States Department of Agriculture and accredited by AAALAC International. The VCU Institutional Animal Care and Use Committee approved all research and enrichment protocols in accordance with the 2011 Guide for the Care and Use of Laboratory Animals.

2.2 Assay of Thermal Nociception

Monkeys were trained to sit comfortably in an acrylic restraint chair using the pole-and-collar technique such that their tails hung freely. The subject's tail was shaved 10-12 cm from the distal end weekly and immersed in a thermal container of warm water. If the subject did not remove its tail by 20 s, the experimenter removed the tail and a latency of 20 s was assigned. A stopwatch was utilized to record tail-withdrawal latencies. During each 15-min cycle, tail-withdrawal latencies were recorded from water warmed to 38°C, 50°C, and 54°C and the order of warmed water presentations was counterbalanced between successive cycles. Baseline tail-withdrawal latencies at all three thermal intensities were determined in each daily test session before drug administration. Test sessions continued only if tail-withdrawal latencies from 38°C water did not occur before the 20 s cutoff. This criterion was met in every monkey during every test session. Time course test sessions consisted of a single drug dose administered intramuscularly (IM) and tail withdrawal latencies were re-determined at 10, 30, and 100 min post-drug administration. Cumulative dose test sessions consisted of four to five 15-min cycles composed of a 10-min drug pretreatment phase and a 5-min testing phase. Drugs were administered IM at the start of each 15-min cycle, and each drug dose increased the total cumulative dose by one-fourth or

one-half log units. Tail-withdrawal latencies were re-determined during the 5-min testing phase as described above.

Initially, the time course of (–)-Ro 64-6198 (0.1 and 0.32 mg/kg) and SB-612111 (0.32 mg/kg) were singly determined. Following these initial Ro 64-6198 time-course experiments, two additional experiments were conducted. First, the effectiveness of Ro 64-6198 to alter the antinociceptive effects of six mu-opioid ligands and the kappa-opioid agonist nalfurafine was determined. Cumulative dose-effect functions for NAQ (0.1-3.2 mg/kg), buprenorphine (0.032-1 mg/kg), nalbuphine (0.032-3.2 mg/kg), morphine (0.1-10 mg/kg), oxycodone (0.01-1 mg/kg), methadone (0.1-5.6 mg/kg), and nalfurafine (0.0001-0.01 mg/kg) were determined following a 30-min pretreatment of 0.1 mg/kg Ro 64-6198 or vehicle. Mu-opioid ligands were tested up to doses that produced maximal antinociception, undesirable effects such as respiratory depression, or reached solubility limits. Nalfurafine was tested up to doses that produced emesis. These experiments were generally conducted twice per week, except for studies with buprenorphine, nalbuphine, and nalfurafine, which were separated by at least six days to allow dissipation of long-acting drug effects and/or to minimize potential effects of antinociceptive tolerance. Ro 64-6198 test sessions were also separated by at least seven days. Second, potency, time course, and antagonism of Ro 64-6198 enhancement of nalbuphine-induced antinociception were determined in three of the four monkeys used for the mu-opioid ligand and Ro 64-6198 interactions described above. One monkey was removed from this set of experiments due to health issues unrelated to the study. These experiments were also separated by at least seven days. The experimenter was not blinded to drug or dose conditions consistent with our previous publications (Banks et al., 2010a; Banks et al., 2010b; Cornelissen et al., 2018a). Ro 64-6198 and vehicle pretreatments were counterbalanced between opioid ligands, but pretreatments were consistent across all monkeys.

2.3 Assay of Schedule-Controlled Responding

Experiments were conducted in each monkey's housing chamber, which also served as the experimental chamber as previously described (Banks et al., 2010b). A custom-fabricated operant response panel and a food pellet dispenser (Med Associates, ENV-203-1000, St. Albans, VT) were attached to the front of the housing chamber. Panels were operated under a MED-PC interface and programmed with a Windows-based computer using MEDSTATE Notation (MED Associates). Training sessions were composed of five 15-min cycles for a total session duration of 75 min. Two components were incorporated into each cycle. The first component was a 10-min time-out period during which responses had no scheduled consequences. The second component was a 5-min response period during which the right key was transilluminated red, and subjects could respond under a fixed-ratio 30 (FR30) schedule of food pellet presentation. If a subject earned the maximum of 10 pellets prior to completion of the 5-min period, the response component was terminated, stimulus lights were extinguished, and further responses resulted in no consequences. All monkeys were trained until rates of responding were 1.0 response/s during all 5 cycles for seven consecutive days (data not shown).

Behavioral sessions were conducted five days per week. Training sessions were conducted on Mondays, Wednesdays, and Thursdays, and test sessions were conducted on Tuesdays and Fridays. Subjects were eligible for participation in test sessions if rates of operant responding were ≥ 1.0 response/s on training days that preceded test days. On test days, test compounds were administered IM using the same cumulative dosing procedure described above in the assay of thermal nociception. All drugs and pretreatment combinations were tested up to doses that either decreased responding $>70\%$ of the preceding training day's average response rate or reached solubility limits. Individual test sessions lasted for 3 to 6 cycles depending on individual subject behavior and treatment condition.

Initially, the potency and time course of vehicle, (–)-Ro 64-6198 (0.1–0.32 mg/kg), and SB-612111 (0.32 mg/kg) were determined. Additionally, the effectiveness of SB-612111 to antagonize the rate-decreasing effects of Ro 64-6198 was evaluated. Subsequently, Ro 64-6198 interactions with the same opioid ligands evaluated in the assay of thermal nociception were determined. Cumulative dose-effect functions for NAQ (0.1–10 mg/kg), buprenorphine (0.032–3.2 mg/kg), nalbuphine (0.032–1 mg/kg), morphine (0.1–5.6 mg/kg), oxycodone (0.01–1.0 mg/kg), methadone (0.1–3.2 mg/kg), and nalfurafine (0.0001–0.0032 mg/kg), were determined following a 30-min pretreatment of 0.1 mg/kg Ro 64-6198 or vehicle. These experiments were generally conducted twice per week, except for studies with buprenorphine, nalbuphine, and nalfurafine, which were separated by at least seven days to allow dissipation of long-acting drug effects and/or to minimize potential tolerance to the rate-decreasing effects of low efficacy mu-opioid ligands. Ro 64-6198 test sessions were also separated by at least seven days. Ro 64-6198 and vehicle pretreatments were counterbalanced between opioid ligands, but pretreatments were consistent across all monkeys.

2.4 Data Analysis

For the assay of thermal nociception, tail-withdrawal latencies (in sec) were converted to percent maximal possible effect (%MPE). %MPE was defined as $\{(\text{Test latency} - \text{Control latency}) \div (20 - \text{Control Latency}) * 100\}$ where “test latency” was the latency in response to either 50°C or 54°C at each dose during the cumulative dosing procedure, and “control latency” was the latency in response to either 50°C or 54°C taken during the baseline period prior to drug administration. Statistical analysis of all %MPE data was conducted using a repeated-measures two-way ANOVA with either time or pretreatment and opioid ligand dose as the main factors (all factors repeated measures). A Sidak or Tukey post-hoc test, as appropriate, followed all significant interactions. Significance was set *a priori* at the 95% confidence level.

For the assay of schedule-controlled responding, rates of operant responding (responses/s) during each test cycle were converted to percent control rate using the average rate of responding of the 5 cycles from the individual monkey's previous training session. Statistical analysis of all % control data was conducted using a repeated-measures two-way ANOVA with either time or pretreatment and opioid ligand dose as the main factors (all factors repeated measures). A Sidak or Tukey post-hoc test, as appropriate, followed all significant interactions.

In addition, the effective dose (ED₅₀) that produced either 50%MPE or 50% reduction in control rates of responding was determined for each mu-opioid ligand and nalfurafine following 0.1 mg/kg Ro 64-6198 or vehicle pretreatment. ED₅₀ values were determined by interpolation when only two data points were available (one below and one >50% effect) or by linear regression when at least three data points on the linear portion of the dose-effect function were available as previously described (Banks et al., 2010b; Cornelissen et al., 2018a; Cornelissen et al., 2018b; Stevenson et al., 2003). Individual ED₅₀ values were subsequently averaged to yield group mean ED₅₀ values and 95% confidence intervals using the Student's T distribution (confidence.t equation in Microsoft Excel for Mac, Version 16.9, Microsoft, Redmond, WA).

2.5 Drugs

(±)-Methadone HCl and (±)-buprenorphine HCl were purchased from a commercial supplier (Spectrum Chemicals, Gardena, CA). (–)-Oxycodone HCl, (–)-morphine sulfate, (–)-nalfurafine HCl, (–)-Ro 64-6198 HCl, and SB-612111 HCl were supplied by the National Institute on Drug Abuse Drug Supply Program (Bethesda, MD). (–)-Nalbuphine HCl was supplied by Dr. Kenner Rice (Drug Design and Synthesis Section, National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism, Bethesda, MD). NAQ HCl was synthesized as previously described (Li et al., 2009) and supplied by Drs. Samuel Obeng and Yan Zhang. Buprenorphine, nalbuphine, oxycodone, morphine, methadone, and nalfurafine were dissolved in sterile water. (–)-Ro 64-6198 was dissolved in a solution of 1:4:5 Tween 80 (Spectrum Chemicals, Gardena, CA) to dimethyl sulfoxide (DMSO) (Sigma-Aldrich, St. Louis, MO) to sterile water. SB-612111 was dissolved in a solution of 4:6 DMSO to sterile water. NAQ was dissolved in a solution of 3:7 DMSO to sterile water. All drug doses were administered intramuscularly and expressed as the salt forms listed above.

3. RESULTS

3.1 Effects of Ro 64-6198 alone

Average ± S.E.M. baseline tail withdrawal latencies for Ro 64-6198 alone and in combination with various mu-opioid ligand experiments were 1.0 ± 0.4 s at 50°C and 0.7 ± 0.1 s at 54°C. Average control rates of responding across all experiments was 2.5 ± 0.4 responses/s. Fig. 1 shows the potency and time course of Ro 64-6198 alone to produce antinociception (panels A and B) and rate-suppression (panel C). Up to 0.32 mg/kg, Ro 64-6198 did not produce significant antinociception with a maximum %MPE of 9.4 ± 7.4 and 1.1 ± 0.8 at 50 and 54°C, respectively. In contrast, Ro 64-6198 produced dose- and time-dependent decreases in rates of responding with maximal effects at 100 min following 0.32 mg/kg administration (treatment: $F_{4,8}=16.2$, $P<0.05$; time: $F_{3,6}=18.8$, $P<0.05$; interaction: $F_{12,24}=2.9$, $P<0.05$). The rate-decreasing effects of Ro 64-6198 were blocked by the NOP antagonist SB-612111 at a dose (0.32 mg/kg) that had no effect on rates of responding alone. Larger Ro 64-6198 doses (1.0 mg/kg) were evaluated, but required prompt SB-612111 administration due to the emergence of undesirable effects including loss of muscle tone and slowed respiratory rate.

3.2 Effects of Ro 64-6198 pretreatment on mu-opioid ligand-induced antinociception

Fig. 2 shows the antinociceptive effects of NAQ, buprenorphine, nalbuphine, morphine, oxycodone, and methadone following either vehicle or 0.1 mg/kg Ro 64-6198 pretreatment. Buprenorphine, nalbuphine, morphine, oxycodone, and methadone produced dose-dependent antinociception at 50°C under vehicle conditions and the corresponding ED₅₀ values for each mu-opioid agonist are reported in Table 1. NAQ produced a group mean $\pm$ S.E.M. maximum %MPE of 4.9 ± 5.1 at 50°C (Fig. 2, panel A). Supplemental Fig. 1 also shows the antinociceptive effects of these same mu-opioid ligands alone at a higher thermal intensity (54°C). Only morphine, oxycodone, and methadone produced > 50%MPE at 54°C and the corresponding ED₅₀ values are reported in Supplemental Table 1. NAQ, buprenorphine, and nalbuphine produced a maximum %MPE of 1.1 ± 1.9 , 9.3 ± 6.9 , and 37.2 ± 7.3 at 54°C, respectively (Supplemental Table 1).

Pretreatment with a Ro 64-6198 dose (0.1 mg/kg) that was ineffective alone significantly enhanced the antinociceptive effects of buprenorphine (dose: $F_{2,6}=29.4$, $P<0.05$; pretreatment: $F_{1,3}=17.4$, $P<0.05$; interaction: $F_{2,6}=17.0$, $P<0.05$), nalbuphine (dose: $F_{4,12}=489$, $P<0.05$; pretreatment: $F_{1,3}=79.3$, $P<0.05$; interaction: $F_{4,12}=19.8$, $P<0.05$), and methadone (dose: $F_{4,12}=56.6$, $P<0.05$; interaction: $F_{4,12}=4.2$, $P<0.05$) at 50°C (Fig. 2). The corresponding ED₅₀ values of each mu-opioid agonist following Ro 64-6198 pretreatment are also reported in Table 1. Post-hoc power analyses indicated the morphine (power=0.65) and oxycodone (power=0.67) experiments were underpowered to detect a significant interaction between Ro 64-6198 and these two mu-opioid agonists for this effect size. At the 54°C thermal stimulus, Ro 64-6198 pretreatment also significantly enhanced the antinociceptive effects of methadone (methadone dose: $F_{4,12}=657$, $P<0.05$; pretreatment: $F_{1,3}=146.4$, $P<0.05$; interaction: $F_{4,12}=65.1$, $P<0.05$). The corresponding ED₅₀ values for methadone and the other mu-opioid agonists at 54°C are also reported in Supplemental Table 1.

3.3 Effects of Ro 64-6198 pretreatment on mu-opioid ligand-induced rate-suppression

Fig. 3 shows the effects of NAQ, buprenorphine, nalbuphine, morphine, oxycodone, and methadone on rates of responding following either vehicle or 0.1 mg/kg Ro 64-6198 pretreatment. NAQ and buprenorphine alone did not significantly alter rates of responding up to the largest doses tested and maximal rate-decreasing effects (mean $\pm$ S.E.M.) were $87.4 \pm 27.9\%$ and $64.9 \pm 20.8\%$ control, respectively (Fig. 3). Larger NAQ and buprenorphine doses could not be examined due to solubility limits. However, NAQ and buprenorphine were tested up to doses that antagonized the antinociceptive effects of other mu-opioid agonists (Cornelissen et al., 2018a; Walker et al., 1995). Nalbuphine, morphine, oxycodone, and methadone alone all produced dose-dependent decreases in rates of responding and the corresponding ED₅₀ values are shown in Table 1. Ro 64-6198 pretreatment did not enhance the effectiveness of NAQ or buprenorphine to alter rates of responding (Fig. 3). Ro 64-6198 pretreatment also did not enhance the potency of nalbuphine, morphine, oxycodone, or methadone to decrease rates of responding as denoted by overlapping confidence limits for ED₅₀ values (Table 1).

3.4 Effects of Ro 64-6198 pretreatment on nalfurafine-induced antinociception and rate-suppression

Fig. 4 shows the antinociceptive (A) and rate-altering (B) effects of nalfurafine following either vehicle or 0.1 mg/kg Ro 64-6198 pretreatment. Nalfurafine alone failed to produce antinociception up to the highest dose tested with maximal effects (mean $\pm$ S.E.M.) of $9.1 \pm 10.7\%$ and $6.8 \pm 2.9\%$ at 50 and 54°C, respectively (Fig. 4 panels A and B). Ro 64-6198 enhanced the antinociceptive effects of nalfurafine at 50°C (nalfurafine dose: $F_{4,8}=4.7$, $P<0.05$; interaction: $F_{4,8}=4.8$, $P<0.05$) (Fig. 4 panel A). Nalfurafine alone produced dose- and time- dependent decreases in rates of responding and the corresponding ED₅₀ values are shown in Table 1. Time course of nalfurafine rate-decreasing effects are shown in Supplemental Fig. 2. Ro 64-6198 pretreatment attenuated the effectiveness of cumulative 0.001 mg/kg nalfurafine to decrease rates of responding (nalfurafine dose: $F_{3,6}=11.7$, $P<0.05$; interaction: $F_{3,6}=8.9$, $P<0.05$). Ro 64-6198 did not alter nalfurafine potency to decrease rates of responding (Table 1).

3.5 Potency, time course, and antagonism of Ro 64-6198 enhancement of nalbuphine antinociception

Fig. 5 shows the potency (A), time course (B), and sensitivity to NOP antagonism (C) of Ro 64-6198 enhancement of nalbuphine antinociception at 50°C. For these experiments, group mean $\pm$ S.E.M. baseline tail withdrawal latencies were 0.8 ± 0.3 s at 50°C and 0.8 ± 0.2 s at 54°C. There was no significant effect of Ro 64-6198 on nalbuphine effects at 54°C (data not shown). Similar to results in Fig. 2, 0.1 mg/kg Ro 64-6198, but not 0.032 mg/kg, enhanced nalbuphine antinociception (nalbuphine dose: $F_{4,8}=39.3$, $P<0.05$; Ro 64-6198 dose: $F_{2,4}=12.1$, $P<0.05$; interaction: $F_{8,16}=8.3$, $P<0.05$) (Fig. 5 panel A). In addition, only the 30-min pretreatment time was sufficient for Ro 64-6198 to enhance the antinociceptive effects of nalbuphine (time: $F_{3,6}=6.3$, $P<0.05$; nalbuphine dose: $F_{4,8}=61.3$, $P<0.05$; interaction: $F_{12,24}=6.8$, $P<0.05$) (Fig. 5 panel B). Finally, Ro 64-6198 enhancement of nalbuphine antinociception was blocked by the NOP antagonist SB-612111 (nalbuphine dose: $F_{4,8}=53.5$, $P<0.05$; pretreatment: $F_{3,6}=11.7$, $P=0.05$; interaction: $F_{12,24}=9.3$, $P<0.05$) (Fig. 5 panel C). SB-612111 (0.32 mg/kg) pretreatment also significantly attenuated the antinociceptive effects of the 0.32 mg/kg cumulative nalbuphine dose (Fig. 5 panel C).

4. DISCUSSION

4.1 Conclusions

The present study determined whether mu-opioid ligand efficacy was a determinant of antinociceptive interactions with the NOP agonist (–)-Ro 64-6198 in rhesus monkeys. There were three main findings. First, both Ro 64-6198 and nalfurafine were more potent to decrease rates of responding than produce antinociception. Second, Ro 64-6198 enhanced the antinociceptive potency of buprenorphine, nalbuphine, and methadone suggesting that mu-opioid agonist efficacy was not a determinant of mu-opioid and NOP agonist interactions. Despite, Ro 64-6198 and mu-opioid agonist interactions displaying some degree of behavioral selectivity, Ro 64-6198 enhancement of mu-agonist antinociception occurred under a narrow range of experimental conditions. Lastly, NOP agonist interactions were not selective for mu-opioid agonists because Ro 64-6198 also enhanced the

antinociceptive effects of the kappa-opioid agonist nalfurafine. Collectively, these results dampen enthusiasm for NOP agonists as candidate “opioid-sparing” adjuncts.

4.2 Effects of Ro 64-6198, mu-opioid agonists, and nalfurafine alone

The mu-opioid agonists buprenorphine, nalbuphine, morphine, oxycodone, and methadone produced dose- and noxious stimulus- dependent antinociception consistent with the extant literature (Cornelissen et al., 2018a; Gatch et al., 1998; Maguire and France, 2014; Walker et al., 1993). Nalfurafine failed to produce antinociception up to a 3-fold larger dose than doses that suppressed rates of responding. Although nalfurafine has been previously shown to produce antinociception in a warm water tail-withdrawal procedure in monkeys (Endoh et al., 2001; Ko and Naughton, 2009), nalfurafine-induced antinociception in these previous studies occurred at doses larger than those that maximally decreased rates of responding in the present study. The NOP agonist Ro 64-6198 also did not produce antinociception up to doses that significantly decreased rates of responding. These results were consistent with a previous monkey study (Saccone et al., 2016), but inconsistent with other monkey studies (Cremeans et al., 2012; Ko et al., 2009; Podlesnik et al., 2011). Reasons for the inconsistent NOP agonist antinociceptive effects in monkeys are not entirely clear and highlight the importance of evaluating candidate analgesics across a broad range of experimental conditions. One potential explanation for the differential Ro 64-6198 antinociceptive results could be related to the experimental and pharmacological histories of the monkeys. For example, monkeys in the Ko, et al (2009) study had not been exposed to any opioid ligands for at least one month prior whereas monkeys in the present study had a more extensive and recent opioid ligand history (Cornelissen et al., 2018a; Cornelissen et al., 2018b). Thus, one interpretation could be that NOP agonists produce antinociception in opioid-naïve or minimally opioid-experienced primates. Although opioid ligand history did not impact the antinociceptive effects of mu-opioid ligands alone in the present study, the degree to which opioid ligand exposure may alter the antinociceptive effects of NOP agonists remains to be empirically determined.

The opioid agonists nalbuphine, morphine, oxycodone, and methadone decreased rates of responding consistent with the extant literature (Banks et al., 2010b; Downs, 1979; Stevenson et al., 2003). The present results extend these findings to the KOR agonist nalfurafine. NAQ failed to significantly alter rates of responding in the present study. Previous studies have shown that NAQ decreases rates of food-maintained responding (Siemian et al., 2016) and to a lesser extent, electrical brain stimulation-maintained responding (Altarifi et al., 2015) in rats suggesting potential species difference in NAQ effectiveness to decrease operant behavior. Buprenorphine also failed to significantly alter rates of responding and these results were consistent with previous buprenorphine results in male monkeys (Negus et al., 2002). Ro 64-6198 rate-decreasing effects in the present study were consistent with previous Ro 64-6198 results in drug discrimination (Saccone et al., 2016) and extended previous findings by determining Ro 64-6198 time course and sensitivity to SB-612111 antagonism. Overall, the behavioral effects of the mu-opioid ligands and nalfurafine alone in the present study provide an empirical foundation for examining interactions with Ro 64-6198.

4.3 Interactions between Ro 64-6198 and mu-opioid or kappa-opioid agonists

Ro 64-6198 significantly enhanced the antinociceptive effects of the mu-opioid ligands buprenorphine, nalbuphine, and methadone as well as nalfurafine. The present results were generally consistent with the direction, but not the magnitude, of previous mu-opioid and NOP agonist antinociceptive interactions with buprenorphine (Cremeans et al., 2012) and morphine (Hu et al., 2010; Ko and Naughton, 2009) in monkeys. NOP agonist enhancement of morphine antinociception has also been reported in mice (Reiss et al., 2008) and rats (Jin-Hua et al., 1997). The present results extended upon these previous findings in three ways. First, NOP and mu-opioid agonist antinociceptive interactions were not dependent upon mu-opioid agonist efficacy. Second, mu-opioid and NOP agonist antinociceptive interactions occurred under a narrow range of experimental conditions such as dose and pretreatment time that suggests limited clinical utility and effectiveness. Lastly, NOP agonist interactions were not selective for clinically effective mu-opioid agonists because Ro 64-6198 also enhanced nalfurafine-induced antinociception.

In contrast to mu-opioid and NOP agonist antinociceptive interactions, Ro 64-6198 did not significantly alter the rate-decreasing effects of any mu-opioid ligand examined. However, Ro 64-6198 significantly attenuated the rate-decreasing effects of cumulative 0.001 mg/kg nalfurafine. These results suggest at least three main conclusions. First, Ro 64-6198 enhancement of mu-opioid agonist antinociception was not due to generalized behavioral depression. However, one caveat is the Ro 64-6198 dose sufficient to enhance mu-opioid agonist antinociception was only 3-fold smaller than the dose that significantly decreased rates of responding. Thus, there may be a potential ceiling for the amount of NOP agonist in the NOP/mu-opioid drug mixture. Second, the present results are consistent with and extend previous NOP and mu-opioid agonist interactions to the mu-opioid agonist undesirable endpoint behavioral depression. Previous studies have reported that NOP agonists do not enhance the respiratory depressant, scratching-behavior, or reinforcing effects of mu-opioid agonists in monkeys (Cremeans et al., 2012; Ko et al., 2009; Podlesnik et al., 2011). Third, although Ro 64-6198 attenuated the rate-decreasing effects of cumulative 0.001 mg/kg nalfurafine, nalfurafine produced maximal rate-depression at similar doses irrespective of pretreatment. Overall, despite mu-opioid and NOP agonist interactions displaying some degree of behavioral selectivity to produce antinociception vs. rate suppression, the magnitude of these interactions were small and not systematic across the various mu-opioid agonists.

4.4 Comparison to mu-opioid Agonist and Other Drug Interactions

Similar to mu-opioid and NOP agonist interactions, cannabinoid receptor agonists, delta-opioid agonists, serotonin uptake inhibitors and serotonin receptor agonists have also produced a selective enhancement of mu-opioid agonist antinociception in rhesus monkeys (Banks et al., 2010b; Gatch et al., 1998; Maguire and France, 2014; Negus et al., 2009; Stevenson et al., 2003). For example, the serotonin uptake inhibitor clomipramine enhanced the antinociceptive effects of low efficacy mu-opioid agonists to a greater extent than high efficacy mu-opioid agonists (Banks et al., 2010b; Gatch et al., 1998). In contrast, cannabinoid agonists enhanced the antinociceptive effects of high efficacy mu-opioid agonists to a greater degree than low efficacy mu-opioid agonists (Maguire and France,

2014). Furthermore, delta agonist enhancement of mu-opioid agonist antinociception did not depend on mu-opioid agonist efficacy similar to the present NOP agonist effects (Negus et al., 2009; Stevenson et al., 2003). Overall, this literature supports 1) the inclusion of multiple dependent measures to assess behavioral selectivity in preclinical analgesia drug development and 2) the systematic evaluation of behavioral interactions with mu-opioid ligands that vary in efficacy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1:

Time course of (-)-Ro 64-6198 and SB-612111 in assays of warm-water tail withdrawal at 50°C (panel A) and 54°C (panel B) and schedule-controlled responding (panel C). Abscissae: time in min after intramuscular Ro 64-6198 administration (log scale). Ordinates: percent maximal possible effect (panels A and B) or percent control rate (panel C). All points represent mean $\pm$ S.E.M. of 4 monkeys (except panel C where $n=3$). Asterisks denote significant ($P<0.05$) difference from vehicle and 0.32 mg/kg (-)-Ro 64-6198 + 0.32 mg/kg SB-612111.

Fig. 2:

Antinociceptive effects (50°C) of NAQ, buprenorphine, nalbuphine, morphine, oxycodone, and methadone following vehicle or 0.1 mg/kg (–)–Ro 64-6198 pretreatment. Abscissae: cumulative intramuscular mu-opioid ligand dose in milligrams per kilogram (log scale). Ordinates: percent maximal possible effect (%MPE). All points represent mean $\pm$ S.E.M. of 4 monkeys (except NAQ and morphine which is n=3). Asterisks denote significant ($P < 0.05$) difference from vehicle.

Fig. 3:

Rate-decreasing effects of NAQ, buprenorphine, nalbuphine, morphine, oxycodone, and methadone following vehicle or 0.1 mg/kg (–)–Ro 64-6198 pretreatment. Abscissae: cumulative intramuscular mu-opioid ligand dose in milligrams per kilogram (log scale). Ordinates: percent control rate. All points represent mean $\pm$ S.E.M. of 3 monkeys. Asterisks points denote significant ($P < 0.05$) difference from vehicle.

Fig. 4: Antinociceptive (50°C; Panel A) and rate-decreasing (Panel B) effects of nalfurafine following vehicle or 0.1 mg/kg (–)-Ro 64-6198 pretreatment. Abscissae: cumulative intramuscular nalfurafine dose in milligrams per kilogram (log scale). Ordinates: percent maximal possible effect (%MPE; panel A) or percent control rate of responding (panel B). Points in panel A represent mean $\pm$ S.E.M. of 4 monkeys and panel B represent mean $\pm$ S.E.M. of 3 monkeys. Asterisks denote significant ($P < 0.05$) difference from vehicle.

Fig. 5: Antinociceptive effects (50°C) of nalbuphine following vehicle, (–)-Ro 64-6198, or SB 612111 pretreatment. Panel A shows effects of different Ro 64-6198 doses. Panel B shows effects of different Ro 64-6198 pretreatment times. Panel C shows sensitivity to the NOP antagonist SB-612111. Abscissae: cumulative intramuscular nalbuphine dose in milligrams per kilogram (log scale). Ordinates: percent maximal possible effect (%MPE). All points

represent mean $\pm$ S.E.M. of 3 monkeys. Asterisks denote significant ($P<0.05$) difference from vehicle. # denote significant difference ($P<0.05$) from Ro 64-6198 + SB-612111.

Table 1:

Opioid ligand ED₅₀ values (95% confidence limits; CL) following vehicle or 0.1 mg/kg (–)-Ro 64-6198 pretreatment in assays of thermal nociception (TW) and schedule-controlled responding (SCR) in male rhesus monkeys. All values represent group mean ED₅₀ values of 3 (SCR) or 4 (TW) monkeys unless otherwise denoted. ^a ED₅₀ values are from three out of four monkeys.

Opioid ligand	TW (50°C) ED ₅₀ in mg/kg (95% CL)	SCR ED ₅₀ in mg/kg (95% CL)
Methadone	1.45 (1.01-1.89)	1.17 (0.66-1.68)
+ Ro 64-6198	0.79 (0.52-1.06)	1.19 (0.63-1.74)
Oxycodone	0.16 (0.02-0.30)	0.24 (0-1.14)
+ Ro 64-6198	0.10 (0-0.45)	0.29 (0.13-0.45)
Morphine (n=3)	3.27 (0-7.76)	0.74 (0-1.89)
+ Ro 64-6198 (n=3)	1.55 (0.86-2.25)	2.17 (0-6.10)
Nalbuphine	0.20 (0.04-0.36)	0.42 (0-1.19)
+ Ro 64-6198	0.05 (0.05-0.07)	0.83 (0-2.88)
Buprenorphine	0.28 (0-0.62) ^a	NC
+ Ro 64-6198	0.06 (0-0.30)	NC
NAQ	NC	NC
+ Ro 64-6198	NC	NC
Nalfurafine (n=3)	NC	0.001 (0.001-0.002)
+ Ro 64-6198 (n=3)	0.006 (0-0.012)	0.002 (0.001-0.014)

NC: not calculable because no drug dose produced >50%MPE or decreased %Control rate below 50%