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Interactions between *Kappa* and *Mu* Opioid Receptor Agonists: Effects of the Ratio of Drugs in Mixtures

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Abstract

Rationale—Pain is the leading reason for seeking health care and *mu* opioid receptor agonists continue to be prescribed despite well-documented adverse effects. *Kappa* opioid receptor agonists have antinociceptive effects with little to no abuse liability and might be useful for treating pain in mixtures. *Kappa:mu* opioid mixtures might be useful if therapeutic effects of each drug can be selectively increased while reducing or avoiding the adverse effects that occur with larger doses of each drug alone.

Objective—This study characterized the effects of the *kappa* opioid receptor agonist spiradoline alone (0.32 – 56 mg/kg) and in 1:10, 1:3, 1:1, and 3:1 mixtures with the *mu* opioid receptor agonists morphine (1.0 – 32 mg/kg) and etorphine (1 – 10 µg/kg) on warm water tail withdrawal latency, body temperature, responding for food, and fecal output in male Sprague Dawley rats (n=24).

Results—Antinociceptive effects were greater than additive for 1:10 and 1:3 spiradoline:morphine mixtures and for 1:10, 1:3, and 1:1 spiradoline:etorphine mixtures. The potency of spiradoline to produce hypothermia was greater with 1:3 and 3:1 spiradoline:etorphine mixtures but not with 1:10 or 1:1 mixtures or with any spiradoline:morphine mixture. The effects of 1:3 spiradoline:morphine on responding for food were additive, whereas 1:1 and 3:1 were greater than additive. Spiradoline did not significantly alter morphine-induced decreases in fecal output.

Conclusions—Overall, mixtures of *kappa* and *mu* opioids might have therapeutic potential for treating pain, particularly when the mixture has a greater ratio of *mu* to *kappa* agonist. If adverse effects of each constituent drug are reduced or avoided, then *kappa:mu* mixtures might be advantageous to *mu* opioids alone.

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Keywords

Opioids; Drug Mixtures; Drug Interactions; Antinociception; Rats

INTRODUCTION

Pain is the leading reason for seeking health care (St. Stauver et al. 2013) and it is estimated that more than 30% of adults in the United States experience recurrent or daily pain (Johannes et al. 2010; Nahil 2015). *Mu* opioid receptor agonists are commonly prescribed to patients with moderate to severe pain despite well-documented adverse effects such as abuse and fatal overdose (Dart et al. 2015; Levy et al. 2015; Volkow and McLellan 2016). Thus, there is a need for drugs that relieve pain with fewer adverse effects than prescription *mu* opioid receptor agonists. *Kappa* opioid receptor agonists have antinociceptive effects in various preclinical models (Briggs et al. 1998; Kivell and Prisinzano 2010; Miaskowski and Levine 1992; Minervini et al. 2017; Ward and Takemori 1982), but unlike *mu* opioid receptor agonists they do not decrease respiration (Howell et al. 1988) or maintain responding in self-administration procedures that are highly predictive of abuse liability in humans (Negus et al. 2008; Woods and Winger 1987). Several laboratories have explored the possibility that *kappa:mu* opioid mixtures might be useful for treating pain without the high risk of abuse and overdose associated with *mu* opioids alone (Dosaka-Akita et al. 1993; Gunther et al. 2017; Negus et al. 2008; Rech et al. 2012; Townsend et al. 2017; Verborgh et al. 1997).

The *kappa* opioid receptor agonist U69,693 in combination with the *mu* opioid receptor agonist fentanyl produces additive antinociceptive effects but not reinforcing effects in rhesus monkeys (Negus et al. 2008), and the *kappa* opioid receptor agonist nalfurafine in combination with the *mu* opioid receptor agonist oxycodone produces additive antinociceptive effects but not reinforcing effects or significant respiratory depression in rats (Townsend et al. 2017). Morphine-induced itching is attenuated by the *kappa* opioid receptor agonists nalfurafine and U50,488 (Ko and Husbands 2009; Sakakihara et al. 2016). Collectively these findings demonstrate that mixtures comprising small doses of *kappa* and *mu* opioid receptor agonists have antinociceptive effects while reducing or avoiding some *mu* receptor-mediated adverse effects. However, *kappa* agonists also have adverse effects (e.g., dysphoria) that have precluded their clinical use (Peters et al. 1987; Pfeiffer et al. 1986) and in the preclinical laboratory doses of *kappa* opioid receptor agonists smaller than those relieving pain can have adverse effects (Dykstra et al. 1987; Gallantine and Meert 2008; Shippenberg et al. 1988). U50,488 alone but not in combination with the *mu* opioid receptor agonist DAMGO impaired motor coordination in a rotarod assay in rats (Sutters et al. 1990), suggesting that *kappa:mu* mixtures might reduce or avoid *kappa* receptor-mediated adverse effects in addition to *mu* receptor-mediated adverse effects; however, this possibility has not been thoroughly investigated.

Kappa:mu opioid mixtures might have an acceptable therapeutic window for treating pain if the potency of each drug to produce therapeutic (antinociceptive) effects is selectively increased while avoiding the adverse effects that occur with larger doses of each drug alone.

The current experiment compared antinociceptive, hypothermic, and rate-decreasing (food-maintained responding) effects of the *kappa* opioid receptor agonist spiradoline alone and in combination with the *mu* opioid receptor agonists morphine and etorphine to test the hypothesis that *kappa:mu* opioid mixtures selectively enhance antinociceptive effects. Previous studies examining *kappa:mu* mixtures focused primarily on antinociceptive effects in the absence of *mu receptor*-mediated adverse effects. The present experiments aimed to extend the existing literature by focusing on the potential of *mu* agonists to reduce the dose of a *kappa* agonist needed for antinociception, as dose-limiting factors currently preclude *kappa* but not *mu* agonists from being used clinically. If *mu* agonists increase the potency of *kappa* agonists for producing antinociception (but not other effects) and if the doses of *mu* agonists in mixtures do not exceed the antinociceptive doses of the *mu* agonists administered alone, then *kappa* agonists in mixtures might be viable therapeutics that warrant a more comprehensive evaluation.

Spiradoline was selected as the *kappa* agonist for the present study both because it has antinociceptive effects when administered alone or with a cannabinoid receptor agonist (Maguire and France 2016; Minervini et al. 2017) and to extend previous studies on *kappa:mu* mixtures to a different *kappa* agonists. Both morphine and etorphine were studied because they represent a lower efficacy and a higher efficacy *mu* agonist, respectively, based on in vivo and in vitro assays (McPherson et al. 2010; Morgan and Christie 2011; Zaki et al. 2000; Walker et al. 1998). Thus, this study directly tested whether differences in efficacy of the *mu* receptor agonist impact interactions with a *kappa* receptor agonist. A warm water tail withdrawal procedure was used to assay antinociception. Body temperature was selected as a dependent variable because *kappa* and *mu* opioid receptor agonists have divergent effects on thermoregulation, with hypothermia occurring after *kappa* receptor activation and hyperthermia occurring after *mu* receptor activation (e.g., Geller et al. 1983). Fecal output was studied because *mu* opioid receptor agonists markedly inhibit gastrointestinal motility and decrease fecal output whereas *kappa* opioid receptor agonists are generally without effect (Porreca et al. 1984, Roger et al. 1994, Tavani et al. 1984). Food-maintained operant responding was used to assess behavioral suppression, a shared effect of *kappa* and *mu* agonists (e.g., Dykstra et al. 1987; Mello and Negus 1998; Negus and Mello 2002). Dose-equivalence and dose-additivity analyses (e.g., Tallarida 2006) were used to quantitatively determine the nature of the interaction between spiradoline and morphine (for antinociception and responding for food) and spiradoline and etorphine (for antinociception).

METHOD

Subjects

Twenty-four male Sprague-Dawley rats (Envigo, Indianapolis, IN) weighed 230–250 g upon arrival and were individually housed in 45 x 24 x 20 cm plastic cages with rodent bedding and free access to water. Rats had free access to standard rodent chow (Envigo Teklad) for one week while habituated to handling. Rats were randomly assigned to one of three groups (n=8 per group); one group was used for antinociception and body temperature experiments, one group was used for operant (food) experiments, and the other group was used for fecal

output experiments. After randomization, all rats were food restricted to 15 ± 5 g daily until they reached a maximum body weight of 375 ± 5 g. Target body weights were maintained by food provided in the home cage after daily sessions. A 14:10 hr light:dark cycle was in effect (lights on at 0630 hr) with sessions conducted during the light period (starting at 1000 and 1400 hr for antinociception and operant studies, respectively, and lasting up to 3 hr). One rat from the antinociception experiment died (unknown reason) prior to completing the study (data not included in analyses), and one rat was removed from the operant (food) responding experiment because after substantial training lever pressing was not maintained above a fixed-ratio 5 schedule of reinforcement. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio and in accordance with guidelines set forth by the Guide for the Care and Use of Laboratory Animals (2011).

Apparatus and Procedure

Warm Water Tail Withdrawal and Body Temperature—Warm water tail withdrawal latency was used to measure antinociceptive effects with body temperature recorded during the same sessions. Water baths (EW-14576-00, Cole-Parmer, Vernon Hills, IL) were maintained at 40, 50, and 55°C. Sessions comprised 6 30-min cycles with an injection given at the start of each cycle; after each injection, the rat was returned to its home cage for 28 min. Next, the rat was gently restrained by the experimenter and 5 cm of the tail was lowered into a water bath. Water temperatures were tested in a randomized order and separated by approximately 20 sec. Latency (sec) to completely withdraw the tail from the water was recorded using a stopwatch (20 sec maximum). At the end of each cycle body temperature was recorded with a rectal thermometer (PhysiTemp Instruments, Inc., Clifton, NJ), the next injection was given, and the rat was returned to its home cage. Saline was administered in the first cycle, and cumulative doses of drug were administered in the remaining 5 cycles, with the stipulation that latencies in the first cycle were not more than 5 sec for 50 and 55°C water and were at least 15 sec for 40°C water. When at least 80% of the maximum possible effect was observed for 50°C water, that test was discontinued. To control for handling and injecting, saline was administered in all cycles of one session.

Drug tests were separated by at least one week. Morphine and spiradoline dose-response curves were determined in an alternating order until dose-response curves were determined twice for each rat. The mean of two determinations was used to construct dose-response functions and obtain ED₅₀ values of each drug for antinociceptive effects (50°C water). The mean ED₅₀ values were used to determine the doses for spiradoline:morphine mixtures in ratios of 10:1, 3:1, 1:1, and 1:3 (see Table 1). The order of testing was determined randomly except for 10:1, which was tested last. When the smallest dose of the 10:1 mixture was tested initially, 4 of 7 rats showed a 60–100% effect (i.e., in Cycle 2); because dose-response functions and ED₅₀ values could not be estimated, the test was discontinued and the dose range was adjusted downward before re-testing the 10:1 mixture (see Table 1). Finally, to assess the generality of the results obtained with morphine to other drugs within the same drug class, the experiment was repeated using the *mu* opioid receptor agonist etorphine. Dose-response curves were determined twice and then averaged to obtain ED₅₀ values for etorphine to produce antinociceptive effects (50°C water) in each rat. The mean ED₅₀ values

were used to determine the doses for spiradoline:etorphine mixtures in ratios of 10:1, 3:1, 1:1, and 1:3 (see Table 1; random order). All dose-response functions for drug mixtures were singly determined.

Food-Maintained Responding—Sessions were conducted in commercially available operant conditioning chambers (31 × 24 × 21 cm; ENV-008CT; Med Associates, Inc.) enclosed in ventilated, sound-attenuating cubicles (ENV-022M; Med Associates, Inc., St Albans, VT). The side panels of the chamber were Plexiglas, and the rear and front panels were aluminum. The front panel was equipped with two response levers horizontally aligned 11.5 cm apart. Above each lever was a 2.5-cm diameter stimulus light that could be transilluminated white with a 100 mA bulb (lever lights). A feeder dispensed 45-mg sucrose pellets (F06233; Bio-Serv, Frenchtown, NJ) to a 5 × 5 cm food aperture centrally located between the two levers. The rear panel was equipped with a 100 mA house light centered 2 cm from the top of the chamber. MED-PC IV software and a PC-compatible interface (Med Associates, Inc.) controlled experimental events and recorded data.

As with the antinociception study, operant sessions comprised 6 30-min cycles with an injection occurring at the start of each cycle. There was a 25-min timeout, during which the chamber was dark (lever presses were recorded but had no programmed consequence), followed by a 5-min response period, during which the house light and left lever light were illuminated. Completing 10 consecutive responses on the left lever (fixed-ratio 10 schedule) resulted in the delivery of a food pellet (signaled with a 0.1-s flash of the house light). If 10 pellets were earned before 5 min elapsed, the house light and the lever light were extinguished for the remainder of the cycle. Drug tests occurred after 3 consecutive non-drug sessions in which overall response rates were within 20% of the mean. For drug tests, saline was administered in the first cycle with cumulative doses of drug administered in the remaining 5 cycles. If the response rate was 20% or less of the saline response rate, subsequent cycles were not conducted. Additionally, there was one session in which saline was administered in all cycles to control for handling and injecting; the criteria for the saline test were the same as for drug tests.

All drug tests were separated by at least one week. Morphine and spiradoline dose-response curves were determined in an alternating order until dose-response functions for each drug were determined twice for each rat. The mean of two determinations was used to obtain ED₅₀ values of each drug to decrease responding for food. The mean ED₅₀ values were used to determine the doses for spiradoline:morphine mixtures in ratios of 3:1, 1:1, and 1:3 (see Table 1). Dose-response functions for drug mixtures were singly determined in a random order. The operant study was completed and those rats were no longer available when the effects of the 1:10 ratio of spiradoline:morphine were assessed in the antinociception study; therefore the 1:10 ratio was not evaluated in the operant study.

Fecal Output—Sessions were conducted in clean home cages (bedding removed) and began with 2 hr access to wet chow (75 g standard chow soaked in 120 ml of warm tap water for approximately 90 min until homogenized). After 2 hr any remaining chow was removed from the cage and weighed; the weight of the remaining chow was subtracted from the initial weight, providing an estimate of consumption. Rats then received an i.p. injection,

and fecal output was collected, counted, and weighed hourly for the next 6 hr. Fecal output was weighed again after 48 hr to determine the dry weight.

All drug tests were separated by at least five days. A morphine dose-response function was determined twice in each rat, first in ascending order and then in descending order. Doses of spiradoline:morphine mixtures (1:10, 1:3, 1:1, and 3:1 ratios) were tested in irregular order, and the doses were the same as those given in the antinociception experiments. Whereas cumulative dosing was used in the antinociception experiments, acute bolus doses were administered for the fecal output experiments.

Drugs

Spiradoline mesylate (Upjohn, Kalamazoo, MI), morphine sulfate (NIDA Drug Supply Program), and etorphine hydrochloride (NIDA Drug Supply Program), and all drug mixtures were dissolved in sterile 0.9% saline solution. Injections were given intraperitoneally (i.p.) in a volume of 1 ml/kg body weight and all doses are expressed in terms of the salt.

Data Analyses

For all antinociception analyses only data from 50°C water are shown because tail withdrawal latencies were nearly exclusively 20 sec for 40°C water and less than 5 sec for 55°C water. The effects of each drug administered alone on tail withdrawal latency (sec), body temperature (°C), and responding for food (responses/sec) were analyzed using a 1-way ANOVA and Dunnett's Multiple Comparison Test (each dose versus the corresponding saline control) with significance levels set to $p < 0.05$.

Tail withdrawal latency was expressed as a percentage of the maximum possible effect according to the following formula: $(\text{Test Latency} - \text{Saline Latency} / 20 - \text{Saline Latency}) * 100$, where control latency corresponds to the level of effect after administration of saline in the first cycle. Rectal body temperature was expressed as an absolute change (°C) from saline. Rate of responding for food was expressed as a percentage of the saline response rate according to the following formula: $(\text{Test Response Rate} / \text{Saline Response Rate}) * 100$. To determine whether the *mu* agonist constituent (morphine or etorphine) altered the potency of the *kappa* agonist constituent (spiradoline) in mixtures, effects of spiradoline on antinociception, body temperature, and responding for food were plotted as a function of the cumulative dose of spiradoline in the mixture (Figure 2). ED₅₀ values of spiradoline alone and in mixtures were obtained for each rat by fitting a regression to the linear portion of the dose-response function (between 20% and 80% effect, including not more than one dose producing less than 20% effect and not more than one dose producing more than 80%). ED₅₀ values (of spiradoline alone versus in a mixture) were then averaged among subjects. If the 95% CIs did not overlap, the potency ratio (ED₅₀ of spiradoline alone / ED₅₀ of spiradoline in a mixture) was considered significant.

The interaction between spiradoline and morphine (on antinociception and food-maintained responding) and the interaction between spiradoline and etorphine (on antinociception) were examined quantitatively using dose-equivalence and dose-additivity analyses as previously described (Tallarida 2006, 2011; dose-equivalence analyses could not be conducted for body temperature, thereby precluding dose-additivity analyses; see Results). For each rat, data

were averaged across two determinations for each drug administered alone, then a regression line was fit to the linear portion of the dose-response function, which encompassed doses ranging from ineffective (20% or less) to effective (80% or more). No more than one dose producing less than 20% effect was included and no more than one dose producing greater than 80% was included. ED₅₀ values for each rat were determined and those values were averaged across the group to provide the basis for the doses used in the mixtures (see Table 1). Based on the ED₅₀, E_{max}, and slope of each drug given alone, the dose of morphine or etorphine in the mixture was converted to spiradoline equivalence for each rat according to the following equation (Grabovsky and Tallarida 2004): $beq(a) = ED_{50} A / [(E_{max} A / E_{max} B) (1 + ED_{50} B^q / b^q) - 1]^{1/p}$ where ED₅₀ A and ED₅₀ B are the doses of drugs A and B estimated to produce a 50% effect, E_{max} A and E_{max} B are the maximum effect levels for drugs A and B, *a* is dose of drug A, and *q* and *p* are the slopes derived from the linear regression analyses of drugs A and B, respectively. The total additive dose (i.e., morphine in spiradoline equivalence plus spiradoline; or etorphine in spiradoline equivalence plus spiradoline) was calculated by adding *beq(a) + a*, and that was used to determine predicted effects (additivity) for individual rats using the following equation (Grabovsky and Tallarida, 2004): Predicted Effect Level = $[E_{max} A (eqA^p)] / [(E_{max} A (eqA^p) + (ED_{50} A^p)]$. Next, a linear regression was fit to all data (i.e., from all rats, not averaged) between the largest dose that produced 20% or less and the smallest dose that produced 80% or more of the predicted effects (additivity) and the observed effects, which were empirically determined. To obtain accurate estimates of the slope of the linear portion of the dose-response function, no more than one dose producing less than 20% effect was included and no more than one dose producing greater than 80% was included for each rat. The slopes and y-intercepts were compared using an F test for each ratio (Table 2), with significance levels set to *p* < 0.05.

Fecal output (count) was expressed as a percentage of the fecal output after saline according to the following formula: (Test Fecal Count / Saline Fecal Count) * 100. The effects of spiradoline:morphine mixtures on fecal output were compared to the effects of mixtures on antinociception by using dose-equivalence to calculate the total morphine equivalent dose (based on antinociception). If the 95% CIs for a mixture did not overlap with the 95% CI for morphine alone, then the effects on fecal output were considered significant. Analyses were conducted using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA).

RESULTS

Tail withdrawal latency (50°C water), body temperature, and rate of responding for food were stable across 6 saline cycles (not shown). For 6 saline cycles, mean (±1 SEM) tail withdrawal latency was 4.8 (0.3) sec, body temperature was 37.2 (0.1) °C, and response rate was 0.78 (0.1) responses per second. For the entirety of these studies, there were no significant differences among the control conditions (i.e., saline in the first cycle) for any of the assays. Mean (±1 SEM) tail withdrawal latencies from 50°C water after saline administration were 4.1 (0.3) sec for the spiradoline control, 4.6 (0.3) sec for the morphine control, and 3.7 (0.2) sec for the etorphine control. Mean (±1 SEM) body temperatures after saline were 37.4 (0.1), 37.3 (0.1), and 37.4 (0.1) °C for the spiradoline, morphine, and etorphine controls, respectively (Fig 1A and 1B, data above “S”). Mean (±1 SEM) response

rates after saline were 0.80 (0.10) and 0.90 (0.10) responses per second for spiradoline and morphine controls, respectively (Fig 1C, data above “S”).

Administered alone each drug increased tail withdrawal latency in a dose-dependent manner (Fig 1A). Spiradoline (10 – 56 mg/kg), morphine (5.6 – 32 mg/kg), and etorphine (1 – 10 µg/kg) significantly increased tail withdrawal latency compared with saline (Fig 1A; $F_{5,31}=41.19$, $p<0.05$ for spiradoline; $F_{5,29}=17.47$, $p<0.05$ for morphine; $F_{5,36}=101.7$, $p<0.05$ for etorphine). Spiradoline (5.6 – 56 mg/kg) significantly decreased body temperature ($F_{5,31}=73.19$, $p<0.05$) whereas morphine (3.2 – 32 mg/kg) and etorphine (3.2 – 10 µg/kg) increased body temperature ($F_{5,30}=73.92$, $p<0.05$ for morphine; $F_{5,36}=7.84$, $p<0.05$ for etorphine) compared with saline (Fig 1B). Spiradoline (1.78 – 3.2 mg/kg) and morphine (3.2 – 10 mg/kg) significantly decreased the rate of responding for food ($F_{5,34}=9.05$, $p<0.05$ for spiradoline; $F_{5,29}=7.13$, $p<0.05$ for morphine) compared with saline (Fig 1C). The potency of spiradoline and morphine to decrease responding for food (Fig 1C) was >10-fold and ~3-fold greater, respectively, than the potency to increase tail withdrawal latency (Fig 1A) and decrease (spiradoline) or increase (morphine) body temperature (Fig 1B).

The absolute doses of spiradoline needed to produce antinociception were decreased 20.8, 8.4, and 2.4-fold when administered with increasing doses of morphine in 1:10, 1:3, and 1:1 ratios, respectively (Figure 2A, left) and 10.1, 8.6, and 5.2-fold when administered with increasing doses of etorphine in 1:10, 1:3, and 1:1 ratios, respectively (Figure 2A, right). The doses of spiradoline needed to produce antinociception were not significantly changed by morphine or etorphine in 3:1 mixtures. The potency of spiradoline to produce hypothermia was greater with 1:3 and 3:1 spiradoline:etorphine mixtures, but not with 1:10 or 1:1 spiradoline:etorphine mixtures (Fig 2B, right) or with any spiradoline:morphine mixture (Fig 2B, left). Spiradoline:morphine in a 1:10 mixture did not produce hypothermia at antinociceptive doses. Smaller absolute doses of spiradoline decreased responding for food when administered with increasing doses of morphine in 1:3 (3.5 fold), 1:1 (2.6 fold), and 3:1 (2.2 fold) ratios compared with spiradoline alone, but these differences were not significant. The doses of morphine and etorphine that decreased the doses of spiradoline needed in mixtures were generally smaller than the doses of these drugs given alone to produce maximal antinociceptive effects (see Online Resource 1). Potency ratios for all mixtures relative to each drug alone are summarized for antinociception and food-maintained responding in Online Resource 2; however, the nature of the interaction cannot be construed from potency ratios alone. Therefore, dose-equivalence and dose-additivity analyses were conducted.

For antinociception, the interaction between spiradoline and morphine (Fig 3A) and between spiradoline and etorphine (Fig 3B) in 1:10 and 1:3 mixtures was greater than additive, as indicated by significant differences in the y-intercepts but not the slopes of the observed results compared with predicted additivity (see Table 2 for statistical analyses). Spiradoline:morphine in 1:1 and 3:1 mixtures (Fig 3A) and spiradoline:etorphine in a 3:1 mixture (Fig 3B) were not significantly different from predicted additivity (Table 2). For spiradoline:etorphine in a 1:1 mixture (Fig 3B), the slope for the observed effects was significantly different (less steep) from the predicted effects (Table 2); thus, the interaction was greater than additive at smaller doses but not at larger doses. For operant (food)

responding (Fig 3C), spiradoline:morphine in a 1:3 mixture was not different from predicted additivity (Table 2). In a 3:1 mixture, the y-intercept but not the slope was significantly greater than additive (Table 2), and in a 1:1 mixture, the slope was significantly different (less steep) from the predicted effects (Table 2), indicating that the interaction was greater than additive at smaller doses but not at larger doses.

The total morphine equivalent doses needed for antinociception were unchanged by spiradoline:morphine mixtures in 1:1 and 3:1 ratios but were smaller with 1:10 and 1:3 ratios compared with morphine alone (Fig 4A), as indicated by a 2-fold leftward shift in the dose-response function. Morphine alone (3.2 – 10 mg/kg) significantly decreased fecal output, and for all spiradoline:morphine mixtures the effects were not significantly different from morphine alone (Fig 4B).

DISCUSSION

Prescription opioids (i.e., *mu* opioid receptor agonists) are widely abused and fatal overdoses have reached epidemic levels (Rudd et al. 2016). Previous studies in rats and monkeys showed that *kappa* opioid receptor agonists and *mu* opioid receptor agonists given in mixtures reduced or avoided the positive reinforcing and respiratory depressant, but not antinociceptive effects, that occur with *mu* agonists alone (Dosaka-Akita et al. 1993; Negus et al. 2008; Townsend et al. 2017; Verborgh et al. 1997). Activation of either *kappa* or *mu* receptors produces antinociception in preclinical models of pain (e.g., Stevenson et al. 2003; Tyers 1980), and because this shared effect involves distinct mechanisms of action, *kappa:mu* mixtures might reduce the dose of each drug needed to relieve pain, thereby diminishing or avoiding adverse effects that occur with larger doses of each drug alone. The present study characterized the antinociceptive, hypothermic, and behavioral suppressant effects of the *kappa* opioid receptor agonist spiradoline alone and in mixtures with the *mu* opioid receptor agonists morphine and etorphine to test the hypothesis that the potency of spiradoline for producing antinociception is selectively increased when administered with a *mu* opioid. The nature of the interaction between spiradoline and morphine and spiradoline and etorphine was examined quantitatively. Additionally, morphine was studied alone and in mixtures with spiradoline for its ability to reduce fecal output.

The major finding in this study was that a 1:10 spiradoline:morphine mixture had antinociception effects with the absolute doses of spiradoline and morphine being more than 20-fold and more than 2-fold smaller, respectively, compared with the doses of each drug administered alone to produce the same level of effect. The interaction for antinociception was greater than additive (synergistic). The 1:10 spiradoline:morphine mixture was without effect on body temperature, and the effect of this mixture on fecal output was not different from the effect of morphine alone. Similarly, for the 1:3 spiradoline:morphine mixture, antinociceptive effects were synergistic, with spiradoline doses being more than 8-fold smaller and morphine doses approximately 3-fold smaller compared with the doses necessary for the same effect when each drug was administered alone. The effects of a 1:3 spiradoline:morphine mixture on food-maintained responding, hypothermia, and fecal output compared were not different from the effects of spiradoline or morphine alone. Moreover, the generality of these findings were extended to a different *mu* opioid receptor

agonist in experiments with spiradoline:etorphine mixtures. Synergistic antinociceptive effects were obtained for the 1:10 mixture and for some doses in the 1:1 mixture, with more than 10-fold (1:10) and more than 5-fold (1:1) decreases in the doses of spiradoline needed for antinociception without corresponding decreases in the doses needed for hypothermic effects. Dose-limiting factors currently preclude *kappa* but not *mu* agonists from being used clinically; therefore, the marked reduction in the doses of spiradoline needed for antinociception (but not other effects) when combined with morphine or etorphine is significant because the doses of *mu* agonists in mixtures did not exceed the antinociceptive doses of the *mu* agonists administered alone. The antinociceptive effects obtained in these studies are not likely due to general sedative effects because the tail withdrawal response occurred rapidly when the intensity of the nociceptive stimulus was increased from 50 to 55°C water (data not shown).

Results of this study clearly demonstrate that the nature of drug-drug interactions for *mu* agonists and *kappa* agonists varies across assays (thermoregulation, food-maintained responding, fecal output, and antinociception) as well as across different ratios of drugs in mixtures. It will be important to determine the nature of interaction between these same classes of drugs for adverse effects that are otherwise very prominent with *mu* agonists (e.g., inhibition of gastrointestinal transit) or *kappa* agonists (e.g., dysphoria) administered alone. This experiment used sensitivity to an acute thermal stimulus (warm water) as a measure of antinociception; however, the type of pain and nociceptive stimulus might also contribute to the nature of the interaction between *kappa* and *mu* agonists. For example, rats treated with the inflammatory agent Freund's complete adjuvant become progressively more sensitive to a mechanical stimulus but not a thermal stimulus over a three-week period (Binder et al. 2000); moreover, antinociceptive effects of the *kappa* agonists asimadoline and PNU50488H depended on the type of nociceptive stimulus. Conversely, the antinociceptive effects of morphine were similar for mechanical and thermal stimuli in mice (Elhabazi et al. 2014).

Morphine and etorphine enhanced a potentially useful effect (antinociception) of spiradoline to a greater extent than hypothermia or behavioral suppression (decreased responding for food). Cannabinoid receptor agonists have also been shown to increase the antinociceptive potency of *mu* opioid receptor agonists, with a greater increase in potency for higher efficacy *mu* agonists (e.g., fentanyl) compared with lower efficacy agonists (e.g., morphine; Maguire and France, 2014). Efficacy of the *mu* agonist did not appear to impact the nature of the interaction for *kappa:mu* mixtures in the current study since etorphine and morphine similarly increased the potency of spiradoline; the antinociceptive effects of 1:10 and 1:3 mixtures were greater than (predicted) additivity with both *mu* agonists. In addition to different drug classes (cannabinoid versus *kappa* opioid) and species (monkey versus rat), the previous study (Maguire and France, 2014) administered a single dose of a cannabinoid receptor agonist prior to cumulative doses of the *mu* opioid agonist, whereas the present study administered cumulative doses of the *kappa* agonist and the *mu* agonist given as a mixture. The temporal relationship between administration of the two drugs (e.g., dosing procedures) in mixtures could also impact the nature of the interaction.

The present data support the notion that *kappa:mu* mixtures might have therapeutic potential for treating pain, particularly when the mixture has a relatively greater ratio of *mu* to *kappa*

agonist. These findings extend previous research on *kappa:mu* mixtures as follows: 1) doses for the mixtures were determined using functionally equivalent doses rather than absolute doses based on the dose-response functions for each drug administered alone; 2) full dose-response functions were generated for all of the drugs administered alone and different *kappa:mu* ratios, thereby allowing for estimation of shifts in potency; 3) the inclusion of physiological endpoints (body temperature and fecal output) in addition to behavioral endpoints; 4) the assessment of interactions with *mu* agonists that differ in their efficacy at the *mu* receptor; and 5) the finding of synergism between *kappa* and *mu* agonists for antinociception. It remains to be determined whether *kappa:mu* mixtures have other adverse effects (e.g., abuse [*mu*], dysphoria [*kappa*]) and whether those effects depend on the particular drugs in the mixture. Additionally, treating pain often requires repeated drug administration, thus it is important to determine whether tolerance or dependence develop during chronic administration of *kappa:mu* mixtures. If adverse effects of each drug are not enhanced in mixtures, then *kappa:mu* opioid mixtures might be advantageous to *mu* opioids alone for treating pain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Tail withdrawal latencies from 50°C water (A) and rectal body temperature (B) after the administration of spiradoline, morphine, or etorphine and operant (lever press) responding for food (C) after the administration of spiradoline or morphine. Ordinates: mean (± 1 SEM) latency in seconds (upper), mean (± 1 SEM) body temperature in °C (middle), and mean (± 1 SEM) response rate in responses per second (lower). Abscissae: cumulative dose in mg/kg (spiradoline and morphine) or µg/kg (etorphine) body weight. The same group of 7 rats contributed to the antinociception and body temperature study and a different group of 7 rats contributed to the operant study.

Figure 2.

Antinociceptive effects (A) and body temperature (B) for spiradoline administered alone (open symbols with dashed line; note that the same data are plotted in the left and right panels) and in mixtures with morphine (left) or etorphine (right) in 1:10, 1:3, 1:1, and 3:1 ratios and response rate for food (C) for spiradoline administered alone and in mixtures with morphine. Ordinates: mean (± 1 SEM) % Maximum Possible Effect (upper), mean (± 1 SEM) change in body temperature from saline control in $^{\circ}$ C (middle), and mean (± 1 SEM) percent of the saline control rate (lower). Abscissae: cumulative spiradoline dose in mg/kg body weight. The same group of 7 rats contributed to antinociception and body temperature study and a different group of 7 rats contributed to the operant study.

Figure 3.

Linear regressions fit to the predicted additive effects and observed (empirically determined) effects for the antinociceptive effects for spiradoline:morphine mixtures (A), antinociceptive effects for spiradoline:etorphine mixtures (B), and food-maintained responding for spiradoline:morphine mixtures (C). Ordinates: mean (± 1 SEM) % Maximum Possible Effect (upper and middle) and mean (± 1 SEM) percent of the saline control rate (lower). Abscissae: cumulative spiradoline equivalent dose in mg/kg body weight. The same group of 7 rats contributed to the antinociception study and a different group of 7 rats contributed to the operant study.

Figure 4.

Linear regressions fit to morphine and spiradoline:morphine mixtures for antinociception (A) and to morphine for fecal output (B). Shaded region denotes the 95% CI for antinociception (upper) and fecal output (lower). Ordinates: mean (± 1 SEM) % Maximum Possible Effect (upper) and mean (± 1 SEM) percent of the saline control fecal output (lower). Abscissae: morphine equivalent dose in mg/kg body weight. A group of 7 rats contributed to the antinociception study and a different group of 8 rats contributed to the fecal output study.

Cumulative doses of spiradolone (mg/kg), morphine (mg/kg), and etorphine (µg/kg) studied in drug mixtures. Doses for the 1:10 spiradolone:morphine mixtures are shown for the adjusted dose range with the initial dose range shown in brackets (see text for details).

Table 1

Antinociception		Spiradolone (mg/kg) : Morphine (mg/kg)					
Cycle		1 : 10	1 : 3	1 : 1	3 : 1		
1		saline	saline	saline	saline		
2	0.13 [1.06]	0.58 [4.64]	1.06	1.39	1.06	0.46	0.46
3	0.26 [2.11]	1.16 [9.29]	2.11	2.79	2.11	0.93	0.93
4	0.53 [4.23]	2.32 [18.58]	4.23	5.57	4.23	1.86	1.86
5	1.06 [8.46]	4.64 [37.15]	8.46	11.15	8.46	3.72	3.72
6	2.11 [16.91]	9.29 [74.30]	16.91	22.29	16.91 ^a	50.73	7.43
		Spiradolone (mg/kg) : Etorphine (µg/kg)					
Cycle		1 : 10	1 : 3	1 : 1	3 : 1		
1		saline	saline	saline	saline		
2	1.06	3.77	1.06	1.13	1.06	0.38	0.38
3	2.11	7.54	2.11	2.26	2.11	0.75	0.75
4	4.23	15.08	4.23	4.52	4.23	1.51	1.51
5	8.46	30.16	8.46	9.05	8.46	3.02	3.02
6	16.91	60.31	16.91	18.09	16.91 ^a	50.73	6.03
Response Rate		Spiradolone (mg/kg) : Morphine (mg/kg)					
Cycle		1:3	1:1	3:1			
1		saline	saline	saline			
2		0.08	0.52	0.08	0.17	0.25	0.17
3		0.17	1.03	0.17	0.34	0.51	0.34
4		0.34	2.06	0.34	0.69	1.01	0.69
5		0.68	4.13	0.68	1.38	2.03	1.38
6		1.35	8.25	1.35 ^b	2.75 ^b	4.05	2.75

^a ED₅₀ of drugs given alone for antinociception (50°C water).

^b ED₅₀ of drugs given alone for food-maintained responding.

Statistical analyses of the predicted (additive) versus the observed effects as shown in Figure 3: spiradoline:morphine and spiradoline:etorphine mixtures for antinociception; and spiradoline:morphine mixtures for response rate (food). The y-intercept denotes the value of y when $\log(x) = 0$.

Table 2

Slope				y-Intercept				
Predicted	Observed	F Test	p	Predicted	Observed	F Test	p	
Antinociception								
Spiradoline:Morphine								
1:10	89.79	97.40	$F_{1,40}=0.17$	0.68	-54.44	-42.34	$F_{1,41}=16.26$	<0.001
1:3	83.06	89.01	$F_{1,40}=0.13$	0.72	-50.57	-33.04	$F_{1,41}=22.50$	<0.001
1:1	91.22	74.42	$F_{1,44}=1.57$	0.22	-60.45	-35.47	$F_{1,45}=1.79$	0.19
3:1	92.97	76.09	$F_{1,43}=0.57$	0.45	-62.67	-37.71	$F_{1,44}=0.83$	0.37
Spiradoline:Etorphine								
1:10	114.20	182.50	$F_{1,39}=3.41$	0.07	-93.92	-159.90	$F_{1,40}=7.36$	<0.01
1:3	112.50	137.50	$F_{1,44}=0.84$	0.37	-89.62	-79.54	$F_{1,45}=38.49$	<0.001
1:1	119.30	64.00	$F_{1,45}=8.25$	<0.01	-96.88	-13.04	n/a	n/a
3:1	113.70	108.10	$F_{1,32}=8.25$	0.86	-91.17	-72.62	$F_{1,33}=2.90$	0.10
Response Rate								
Spiradoline:Morphine								
1:3	-109.00	-97.74	$F_{1,32}=0.10$	0.76	57.91	66.88	$F_{1,33}=1.00$	0.33
1:1	-103.60	-34.77	$F_{1,34}=4.30$	0.04	64.89	39.48	n/a	n/a
3:1	-112.40	-75.73	$F_{1,36}=1.57$	0.22	64.64	41.06	$F_{1,37}=6.76$	0.01