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Effects of Direct- and Indirect-Acting Serotonin Receptor Agonists on the Antinociceptive and Discriminative Stimulus Effects of Morphine in Rhesus Monkeys

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Serotonergic (5-HT) systems modulate pain, and drugs acting on 5-HT systems are used with opioids to treat pain. This study examined the effects of 5-HT receptor agonists on the antinociceptive and discriminative stimulus effects of morphine in monkeys. Morphine increased tail-withdrawal latency in a dose-related manner; 5-HT receptor agonists alone increased tail-withdrawal latency at 50 °C but not 55 °C water. The antinociceptive effects of morphine occurred with smaller doses when monkeys received an indirect-acting (fenfluramine) or direct acting (8-OH-DPAT, F13714, buspirone, quipazine, DOM, and 2C-T-7) agonist. The role of 5-HT receptor subtypes in these interactions was confirmed with selective 5-HT_{1A} (WAY100635) and 5-HT_{2A} (MDL100907) receptor antagonists. None of the 5-HT drugs had morphine-like discriminative stimulus effects; however, fenfluramine and 5-HT_{2A} receptor agonists attenuated the discriminative stimulus effects of morphine and this attenuation was prevented by MDL100907. The 5-HT_{1A} receptor agonists did not alter the discriminative stimulus effects of morphine. Thus, 5-HT receptor agonists increase the potency of morphine in an assay of antinociception, even under conditions where 5-HT agonists are themselves without effect (ie, 55 °C water), without increasing (and in some cases decreasing) the potency of morphine in a drug discrimination assay. Whereas 5-HT_{2A} receptor agonists increase the potency of morphine for antinociception at doses that have no effect on the rate of operant responding, 5-HT_{1A} receptor agonists increase the potency of morphine only at doses that eliminate operant responding. These data suggest that drugs acting selectively on 5-HT receptor subtypes could help to improve the use of opioids for treating pain.
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INTRODUCTION

Pain is one of the most common symptoms of patients and, particularly when pain is moderate or severe, it is typically treated with opioids (eg, hydrocodone); however, the use of opioids for treating pain is limited both by unwanted effects (eg, constipation, abuse) and by the ineffectiveness of opioids in some patients (Gutstein and Akil, 2005). In an attempt to improve the effectiveness of opioids, both from the perspective of decreasing unwanted effects and from the perspective of enhancing therapeutic effects, they have been administered in combination with other (non-opioid) drugs. This strategy has the possibility to increase the overall effectiveness of opioids and to decrease the dose of opioid necessary for treating pain. Decreasing the dose of opioid (eg, in a drug combination) might also decrease the occurrence of adverse effects, particularly during long-term treatment. A drug combination (Vicodin) that is used widely for treating moderate or severe pain includes a μ-opioid receptor agonist (ie, hydrocodone) and a non-opioid (acetaminophen). Although this combination can exert greater antinociceptive effects when compared with either drug administered alone (Malmberg and Yaksh, 1993; Miranda *et al*, 2007), it has a high potential for abuse and for the development of physical dependence (Manchikanti, 2007).

Serotonergic (5-HT) systems can modulate pain, and drugs acting on 5-HT systems (eg, selective serotonin reuptake inhibitors (SSRIs]) are often used in combination

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with opioids for treating pain, reportedly because indirect-acting 5-HT receptor agonists enhance the antinociceptive effects of opioid receptor agonists. For example, the SSRI fluoxetine potentiates the antinociceptive effects of morphine in rats and rhesus monkeys (Larson and Takemori, 1977; Hynes *et al*, 1985; Gatch *et al*, 1998), and the 5-HT releaser fenfluramine increases the analgesic effects of morphine in humans (Coda *et al*, 1993). Indirect-acting agonists increase the extracellular concentration of neurotransmitter that can act on a variety of receptors. As many as 14 different 5-HT receptor subtypes have been identified, although the role of particular subtypes in modulating the antinociceptive effects of opioids is not known. It is clear that agonist activity at certain 5-HT receptor subtypes can modulate nociception. For example, the 5-HT_{1A} receptor agonist 8-hydroxy-*N,N*-dipropyl-2-aminotetralin (8-OH-DPAT) has antinociceptive effects in mice (Fasmer *et al*, 1986) and rats (Crisp *et al*, 1991), and the SSRI clomipramine enhances the antinociceptive effects of opioids in monkeys (Banks *et al*, 2010). To the extent that particular 5-HT receptor subtypes mediate the enhancement of opioid antinociception, new drugs might be developed that target those receptors, thereby possibly further improving pain treatment with drug combinations.

This study examined the effects of indirect- and direct-acting 5-HT receptor agonists on the antinociceptive and discriminative stimulus effects of morphine. Because some agonists enhanced the potency of morphine in producing antinociceptive effects, the generality of that enhancement to other effects was examined by studying the ability of the same 5-HT receptor agonists to modify the discriminative stimulus effects of morphine; drug discrimination procedures are pharmacologically selective and they can be predictive of abuse potential in humans (Schuster and Johanson, 1988). The 5-HT releaser fenfluramine was studied because it increases the analgesic effects of morphine in humans (Coda *et al*, 1993). Drugs with agonist activity at 5-HT_{1A} or 5-HT_{2A} receptors were studied both because these receptors appear to have a role in modulating pain (Xu *et al*, 1994; Crisp *et al*, 1991) and because these receptors are thought to mediate some behavioral effects of fenfluramine (McCreary *et al*, 2003). The 5-HT_{1A} receptor agonists studied were selected because they have been shown to vary in efficacy at 5-HT_{1A} receptors (ie, F13714 > 8-OH-DPAT > buspirone; Koek *et al*, 2001).

MATERIALS AND METHODS

Animals

Four adult rhesus monkeys (*Macaca mulatta*; one male, three female) were used for thermal nociception studies and four other adult rhesus monkeys (two male, two female) were used for drug discrimination studies. Monkeys weighed between 5 and 9 kg, all had received drugs in previous studies, and they received chow (Harlan Teklad High Protein Monkey Diet, Madison, WI), fresh fruit, and peanuts after daily sessions. Monkeys were individually housed on a 14/10-h light/dark cycle with unlimited access to water. The monkeys used in these studies were maintained in accordance with the institutional animal care and use committee, University of Texas Health Science Center at San Antonio, and with the 1996 Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources on Life Sciences, National Research Council, National Academy of Sciences).

Apparatus

For antinociception and drug discrimination studies, monkeys were seated in commercially available primate chairs (Model R001, Primate Products, Miami, FL) that provided restraint. Monkeys in drug discrimination studies were placed in ventilated, sound-attenuating chambers equipped with two response levers and stimulus lights located above each lever. Feet were placed into shoes containing brass electrodes to which a brief (250 ms, 3 mA) electric shock could be delivered from a remote AC generator. Experiments were controlled and data were recorded with a personal computer and a commercially available interface (Med Associate, St Albans, VT).

Thermal Nociception

The warm water tail-withdrawal procedure is described in detail elsewhere (Li *et al*, 2007). Briefly, monkeys were seated in chairs and the lower portion ($\sim$15 cm) of the shaved tail was immersed in a thermal flask containing 40, 50, or 55 °C water. Response to the three temperatures was examined every 15 min (ie, the duration of each cycle); the order of testing different temperatures varied nonsystematically among monkeys and across cycles. When a subject failed to remove its tail from water within 20 s, the experimenter removed the thermos and a latency of 20 s was recorded. Test sessions began with control (no drug) determinations for each temperature. For each cycle, tail-withdrawal latencies were measured at each of the three temperatures with $\sim$1 min between tests and an interinjection interval of 15 min (ie, the same interinjection interval used in the drug discrimination study). On different occasions, the effects of different doses of test compounds were assessed alone and in combination with increasing doses of morphine administered s.c. during the first min of consecutive cycles (test drugs were administered 20 min before the first dose of morphine). A test was terminated for an individual when the maximal effect (20 s) was obtained with 50 °C water.

Morphine Discrimination

Four monkeys were trained previously to discriminate between saline and morphine in a multiple-cycle, cumulative-dosing procedure (Li *et al*, 2008). Each cycle comprised a 10-min timeout, during which the chamber was dark and responses had no programmed consequence, followed by a 5-min response period, during which illumination of red lights signaled a pending electric stimulus (15 s). Five consecutive responses (fixed ratio (FR) 5) on the correct lever extinguished the red lights and postponed delivery of the scheduled electric stimulus for 30 s. Responses on the incorrect lever reset the response requirement on the correct lever. The correct lever was determined by an injection (eg, left, saline; right, morphine) during the first min of the cycle; designation of correct levers varied among

monkeys and remained the same for an individual throughout the study. Response periods ended after 5 min or after the delivery of four electric stimuli, whichever occurred first.

For saline training sessions, saline or a sham injection was administered s.c. during the first min of each of 2–8 cycles. For morphine training sessions, 1.78 mg/kg of morphine was administered s.c. during the first min of a cycle followed by a saline or sham injection during the first min of one subsequent cycle; only the morphine-appropriate lever was active during both of these cycles. On some training days, 2–6 saline or sham training cycles preceded the administration of morphine. Monkeys had previously satisfied the following criteria for testing: 5 consecutive or 6 of 7 days in which at least 80% of the total responses occurred on the active (correct) lever and <5 responses (one FR) occurred on the incorrect (inactive) lever before completion of the FR on the correct lever. For the current study, these criteria had to be satisfied for two consecutive sessions (one morphine training session and one saline training session) before each test. The type of training session immediately preceding a test varied nonsystematically. Test sessions were identical to training sessions except that five consecutive responses on either lever postponed the stimulus schedule and animals received various drugs in combination with cumulative doses of morphine. During test sessions, saline or vehicle was administered in the first cycle followed by increasing doses of morphine in subsequent cycles up to doses that occasioned at least 80% responding on the morphine-appropriate lever, resulted in delivery of an electric stimulus, or to a cumulative dose of 10 mg/kg. Test drugs were administered 5 min before the first cycle (ie, 20 min before the first injection of morphine).

Data Analyses

For the antinociception study, tail-withdrawal latency was expressed as a percentage of the maximal possible effect (MPE) as follows: % MPE = ((test latency–control latency)/(20 s–control latency)) × 100 (control latency was determined in the absence of drug). The MPE was calculated for each individual and then averaged among four monkeys. Linear regression was used to estimate the potency to produce 80% (50 °C) or 25% (55 °C) of the MPE (ie, ED_{80} and ED_{25}, respectively) for morphine administered alone and in combination with 5-HT receptor agonists. These values were determined for individuals and then averaged among four monkeys in a group. ED_{80} values were used with 50 °C water because in some cases the 5-HT agonist or the 5-HT agonist in combination with the smallest dose of morphine produced $>50\%$ MPE. ED_{25} values were used with 55 °C water because in some cases the maximum effect obtained with morphine was $<50\%$ MPE. To further examine the nature of interaction between 5-HT receptor agonists and morphine, ED_{80} values (50 °C only) were also determined for 5-HT receptor agonists administered alone by constructing dose-response curves from data obtained during the first cycle of tests when a 5-HT agonist was administered before increasing doses of morphine. When the largest dose studied of a 5-HT receptor agonist failed to produce at least 80% MPE, it was assumed that the next largest dose produced a full (100%) effect; this assumption provided an estimate of the potency of 5-HT receptor agonists. For antinociception data, parallelism of dose-response curves (drugs alone and in combination) was analyzed by an F-ratio test ($P<0.05$).

Isobolograms were constructed to examine whether the effects of drug combinations were additive, supra-additive, or infra-additive (see, eg, Li *et al*, 2010). An isobologram plots equieffective doses (eg, ED_{80}) of one drug in the presence of different doses of a second drug. If the effects of the two drugs are additive, then the ED_{80} values (±SEM) for the drug combination should overlap with the diagonal line between the ED_{80} values (±SEM) for the two drugs alone (line of additivity). If the ED_{80} values (±SEM) fall below the limits of the line of additivity, then the effects of the two drugs are considered to be supra-additive (ie, in the presence of one drug, smaller than predicted doses of a second drug are needed to produce the same effect). If the ED_{80} values (±SEM) fall above the limits of the line of additivity, then the effects of the two drugs are considered to be infra-additive (ie, in the presence of one drug, larger than predicted doses of a second drug are needed to produce the same effect).

For the drug discrimination study, the following two dependent variables were measured: the percentage of responses on the morphine-appropriate lever during the response period, and the rate of responding during the response period in responses per second. The mean percentage of responses on the drug-appropriate lever ±1 SEM and the mean rate of responding ±1 SEM during test sessions were plotted as a function of dose. Potencies were estimated for the dose required to generate 50% responding on the morphine-appropriate lever (ED_{50}), along with 95% confidence limits (CLs), using linear regression. For some doses of 5-HT receptor agonists, the morphine discrimination dose-response curve was shifted down and the largest dose of morphine (10.0 mg/kg) produced $<50\%$ effect; in order to conservatively estimate the shift to the right in the morphine dose-response curve, it was assumed that the next largest dose of morphine (17.8 mg/kg) produced a maximum effect (100% morphine-appropriate responding).

Dose ratios (ED_{80} or ED_{25} for antinociception and ED_{50} for discrimination) were calculated to estimate the magnitude of shift in the morphine dose-response curve produced by 5-HT receptor agonists. These values were determined for individuals and then averaged among four monkeys in a group. When the 95% CLs of the mean dose ratio did not encompass 1, the morphine dose-response curve was considered to be shifted significantly left (antinociception) or right (discrimination).

Drugs

The compounds used included the following: morphine sulfate, 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane (DOM), and 2,5-dimethoxy-4-*n*-propylthiophenethylamine (2C-T-7), all provided by the National Institute on Drug Abuse (Research Technology Branch, Rockville, MD); MDL100907 was synthesized as described previously (Ullrich and Rice, 2000); (±) fenfluramine hydrochloride, 8-hydroxy-2-(di-n-propylamino) tetralin hydrochloride (8-OH-DPAT), quipazine maleate salt, and buspirone

hydrochloride were purchased from Sigma-Aldrich (St Louis, MO); 3-chloro-4-fluorophenyl-(4-fluoro-4-([(5-methyl-6-methylaminopyridin-2-ylmethyl) amino) methyl] piperidin-1-yl) methanone fumaric acid salt (F13714) and *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-2-pyridinylcyclohexanecarboxamide maleate salt (WAY100635) were gifts from Dr Adrian Newman-Tancredi (Centre de Recherche Pierre Fabre, Castres, France). MDL100907 was dissolved in 20% dimethyl sulfoxide (v/v) and saline; other drugs were dissolved in sterile 0.9% saline. Doses are expressed as the forms listed above in mg per kg of body weight; drugs were administered s.c. in volumes of 0.1–1.0 ml.

RESULTS

Thermal Nociception

Under control conditions, monkeys did not remove their tails from 40 °C water within 20 s (ie, a maximum response of 20 s was recorded) but rapidly removed their tails from 50 and 55 °C water; the average latency (mean ± SEM) for monkeys to remove their tails from 50 and 55 °C water was 2.47 ± 1.08 and 0.91 ± 0.08 s, respectively. Morphine dose dependently increased the latency for monkeys to remove their tails from 50 or 55 °C water (circles, Figure 1; ED_{50} 1.98 (95% CL 1.24, 3.16) mg/kg for 50 °C and ED_{50} 3.95 (95% CL 2.93, 5.23) mg/kg for 55 °C). A dose of 5.6 mg/kg morphine produced 100% MPE for 50 °C water and 56.5 ± 22.0% MPE for 55 °C water.

Administration of 1.0 or 3.2 mg/kg fenfluramine alone had no effect on tail-withdrawal latency (data not shown), whereas administration of 10 mg/kg increased tail-withdrawal latency to 48% MPE for 50 °C water without affecting latency for 55 °C water (triangles above 'V', Figure 1). Fenfluramine also enhanced the effects of morphine on tail-withdrawal latency with a dose of 10 mg/kg shifting the morphine dose-response curve 6.4- and 2.9-fold to the left for 50 and 55 °C water, respectively (triangles, Figure 1). A dose of 0.01 mg/kg of the 5-HT_{2A} receptor antagonist MDL100907 reversed fenfluramine-induced enhancement of the effects of morphine and prevented the shift leftward of the morphine dose-response curve (diamonds, Figure 1). In contrast, a dose of 0.1 mg/kg of the 5-HT_{1A} receptor antagonist WAY100635 in combination with 10 mg/kg of fenfluramine shifted the morphine dose-effect curve further to the left (when compared with 10 mg/kg fenfluramine and morphine) for both 50 and 55 °C water (squares, Figure 1).

When administered alone, F13714, 8-OH-DPAT, and buspirone increased tail-withdrawal latency for 50 °C but not for 55 °C water. Doses of 0.032 mg/kg F13714, 0.32 mg/kg 8-OH-DPAT, and 1.0 mg/kg buspirone produced 30–74% MPE for 50 °C water without markedly affecting tail-withdrawal latency for 55 °C water (inverted triangles above 'V', Figure 2). When administered in combination with each of these 5-HT_{1A} receptor agonists, smaller doses of morphine were effective at both 50 and 55 °C water (inverted triangles, Figure 2). Shifts leftward (mean and 95% CL) in the morphine dose-response curve (estimated by dose ratios for ED_{80} and ED_{25} values for 50 and 55 °C water, respectively) were significant for 50 °C but not for 55 °C water as follows: 2.9 (1.0, 4.5) and 1.7 (0.3, 2.0) for 0.032 mg/kg F13714; 7.0 (3.6, 10.3) and 2.7 (0.9, 4.4) for 0.32 mg/kg 8-OH-DPAT; and 2.3 (1.1, 3.5) and 1.6 (0.9, 2.3) for 1.0 mg/kg buspirone. Shifts leftward in the morphine dose-response curve that were observed in the presence of 5-HT_{1A} receptor agonists were partially or fully prevented by a dose of 0.1 mg/kg WAY100635 (diamonds, all panels, Figure 2).

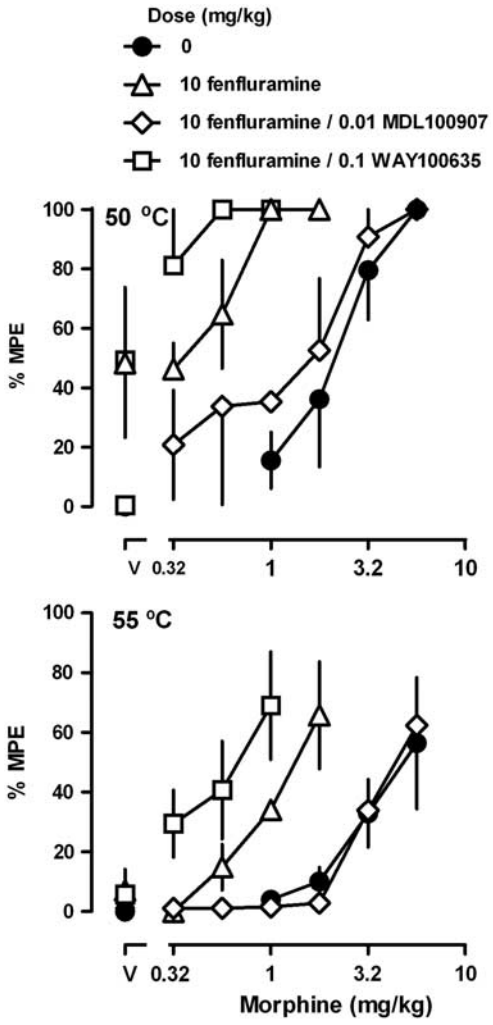

Figure 1 Effects of morphine alone, in combination with the 5-HT releaser fenfluramine, and in combination with fenfluramine as well as either MDL100907 (5-HT_{2A} receptor antagonist) or WAY100635 (5-HT_{1A} receptor antagonist) on tail-withdrawal latency from 50 °C (upper) and 55 °C (lower) water in four rhesus monkeys. Ordinates: average (± SEM) tail-withdrawal latency expressed as a percentage of the maximal (ie, 20 s) possible effect (% MPE). Abscissa: dose of morphine in mg per kg body weight; 'V' is vehicle.

When administered alone, DOM, 2C-T-7, and quipazine increased tail-withdrawal latency for 50 °C but not 55 °C water. Doses of 0.32 mg/kg DOM, 0.032 mg/kg 2C-T-7, and 3.2 mg/kg quipazine produced 22–38% MPE for 50 °C water without markedly affecting tail-withdrawal latency for 55 °C water (Figure 3). When administered in combination with each of these 5-HT_{2A} receptor agonists, smaller doses of morphine were effective at both 50 and 55 °C water (Figure 3). Shifts leftward in the morphine dose-response curve (for 50 and 55 °C water, respectively) were significant

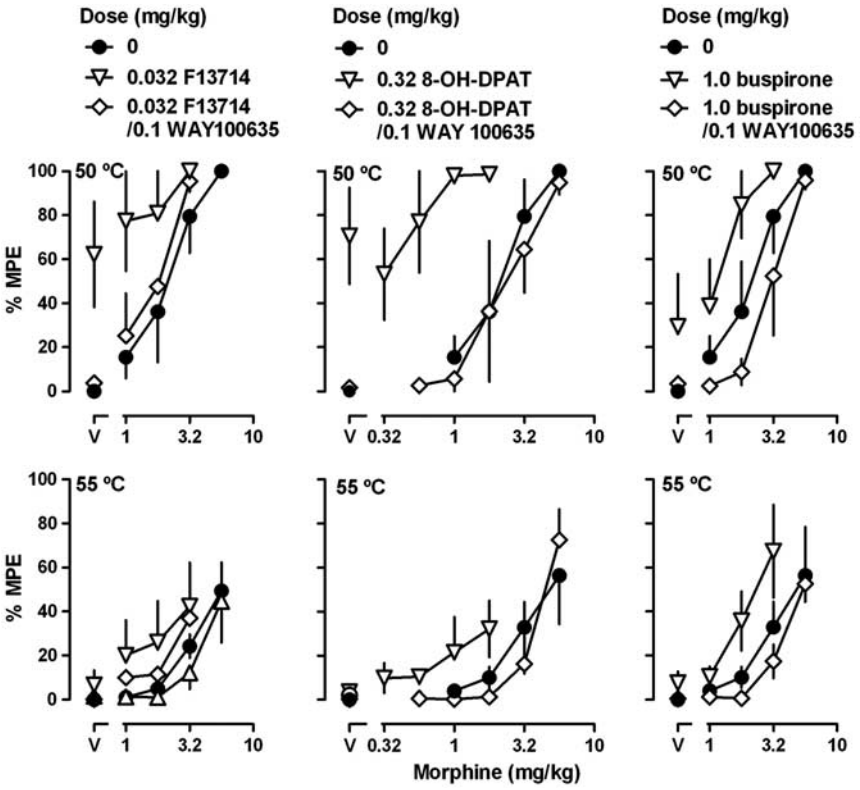

Figure 2 Effects of morphine alone, in combination with the 5-HT_{1A} receptor agonists F13714, 8-OH-DPAT, or buspirone, and in combination with F13714, 8-OH-DPAT, or buspirone as well as WAY100635. See Figure 1 for other details.

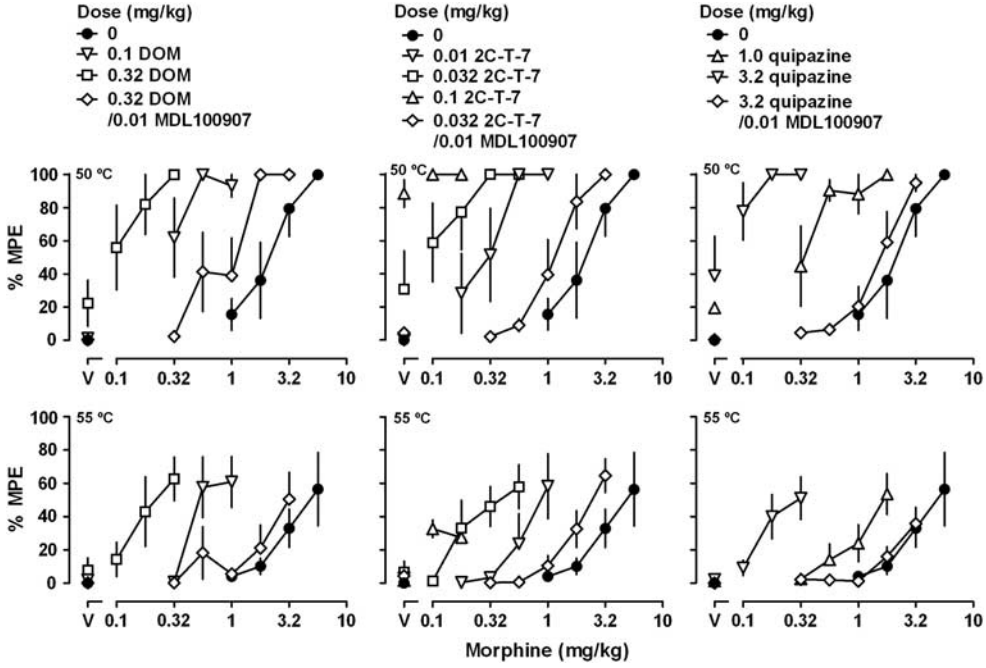

Figure 3 Effects of morphine alone, in combination with the 5-HT_{2A} receptor agonists DOM, 2C-T-7, or quipazine, and in combination with DOM, 2C-T-7, or quipazine as well as MDL100907. See Figure 1 for other details.

for 50 and 55 °C water as follows: 23.0 (14.5, 31.6) and 18.9 (13.3, 24.6) for 0.32 mg/kg DOM; 23.7 (10.8, 36.6) and 13.6 (8.2, 19.0) for 0.032 mg/kg 2C-T-7; and 29.3 (16.5, 42.0) and 17.7 (12.9, 22.4) for 3.2 mg/kg quipazine. Shifts leftward in the morphine dose-response curve, which were observed in the presence of 5-HT_{2A} receptor agonists, were partially or fully prevented by a dose of 0.01 mg/kg MDL100907 (diamonds, Figure 3).

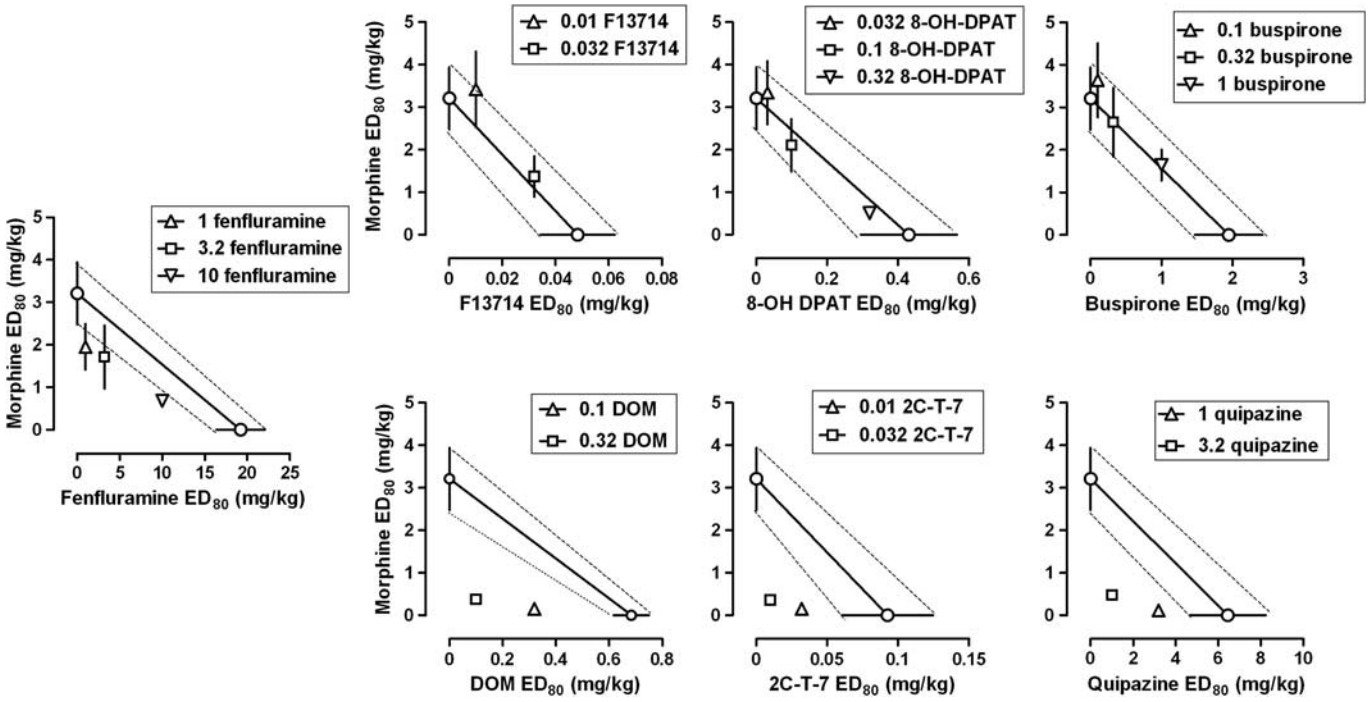

Figure 4 Isobolographic plots of morphine in combination with one indirect-acting (fenfluramine) and six direct-acting (F13714, 8-OH-DPAT, buspirone, DOM, 2C-T-7, and quipazine) 5-HT receptor agonists for antinociceptive effects at 50 °C water. Ordinates: ED_{80} (mg/kg) of morphine. Abscissae: ED_{80} of 5-HT receptor agonists. The diagonal solid line indicates the line of additivity and the diagonal dashed lines indicate 1 SEM from the line of additivity.

ED_{80} values, derived from the data obtained with 50 °C water that are shown in Figures 1–3, were used to plot isobolograms in Figure 4. Tests for parallelism failed to show any significant difference among the slopes of dose-response curves (morphine alone and morphine in combination with 5-HT receptor agonists). ED_{80} values for combinations of the 5-HT_{1A} receptor agonists are within 1 SEM (dashed lines) of the diagonal line connecting ED_{80} values for morphine alone and for each 5-HT_{1A} receptor agonist alone, indicating that the interaction between morphine and each of these agonists was not different from additivity. In contrast, ED_{80} values for combinations of morphine and fenfluramine as well as morphine and each of the 5-HT_{2A} receptor agonists are not within 1 SEM of the line of additivity. The location of these data points (squares and triangles) in each isobologram (ie, to the left and below the diagonal line connecting ED_{80} values for morphine alone and for each 5-HT_{2A} receptor agonist alone), particularly for 5-HT_{2A} receptor agonists, indicates a supra-additive interaction between morphine and each of these agonists (Figure 4).

Morphine Discrimination

Morphine dose dependently increased responding on the morphine-appropriate lever; a dose of 3.2 mg/kg occasioning >90% responding on the morphine lever (circles, upper panel, Figure 5; ED_{50} 0.76; 95% CL 0.56, 1.03). Up to a dose of 3.2 mg/kg, fenfluramine occasioned responding exclusively on the vehicle-appropriate lever (points above 'V', upper panel, Figure 5); however, these doses of fenfluramine attenuated the discriminative stimulus effects of morphine, shifting the morphine dose-response curve to the right and down. Shifts to the right in the morphine discrimination dose-response curve were significant for 3.2 mg/kg (dose ratio = 15.2; 95% CL 10.5, 19.8) but not 1.0 mg/kg (dose ratio = 4.5; 95% CL 0.3, 8.6) fenfluramine. Under control conditions, monkeys responded exclusively on the drug-appropriate lever after receiving 3.2 mg/kg morphine; in contrast, when 3.2 mg/kg fenfluramine was administered before the session, monkeys responded exclusively on the vehicle-appropriate lever after receiving 3.2 mg/kg morphine (Figure 5). Attenuation of the discriminative stimulus effects of morphine by 3.2 mg/kg of fenfluramine was partially prevented by a dose of 0.01 mg/kg MDL100907 (compare inverted triangles and diamonds, upper panel, Figure 5). Response rate was not markedly altered by morphine alone or in combination with fenfluramine; rate was slightly decreased when morphine was combined with both fenfluramine and MDL100907 (diamonds, lower panel, Figure 4).

Monkeys responded predominantly on the vehicle-appropriate lever after receiving F13714, 8-OH-DPAT, or buspirone (points above 'V', upper panels, Figure 6). Pretreatment with the largest dose of each 5-HT_{1A} agonist, which did not eliminate responding when administered alone, shifted the morphine dose-response curve slightly, but not statistically significant, to the right as follows (mean dose ratio (95% CL)): 1.8 (0.7, 2.8) for 0.01 mg/kg F13714; 2.5 (0.3, 4.9) for 0.1 mg/kg 8-OH-DPAT; and 4.0 (−1.2 to 9.4) for 0.32 mg/kg buspirone.

Monkeys responded exclusively on the vehicle-appropriate lever after receiving DOM, 2C-T-7, or quipazine (points above V, upper panels, Figure 7). Pretreatment with doses of each 5-HT_{2A} agonist, which had no effect on rate of responding, shifted the morphine dose-response curve to the

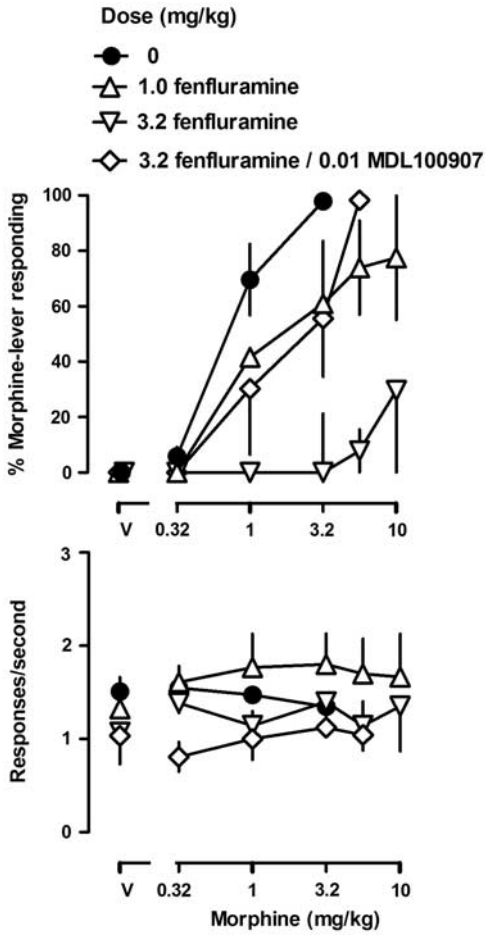

Figure 5 The discriminative stimulus effects of morphine alone, in combination with the 5-HT releaser fenfluramine, and in combination with fenfluramine and the 5-HT_{2A} receptor antagonist MDL100907 in four rhesus monkeys discriminating 1.78 mg/kg morphine from saline. Ordinates: upper, average percentage of responses on the morphine-appropriate lever (±SEM); lower, average rate of lever pressing (±SEM) expressed as responses per second. Abscissae: dose of morphine in mg per kg of body weight' 'V' is vehicle.

right as follows: 6.7 (1.1, 12.6) and 11.6 (6.9, 16.3) for 0.1 and 0.32 mg/kg DOM, respectively; 5.8 (−1.4 to 13.0) and 8.5 (2.2, 14.7) for 0.01 and 0.032 mg/kg 2C-T-7, respectively; and 7.8 (−1.4 to 16.9) for 1.0 mg/kg quipazine. In the presence of the next larger dose of quipazine, 3.2 mg/kg, monkeys responded predominantly on the vehicle-appropriate lever up to a dose of 10 mg/kg (inverted triangle, upper right panel, Figure 7). Attenuation of the discriminative stimulus effects of morphine by DOM, 2C-T-7, and quipazine was largely prevented by 0.01 mg/kg MDL100907 (diamonds, upper panels, Figure 6). Response rate was not markedly affected by morphine alone or in combination with these doses of DOM, 2C-T-7, quipazine and MDL100907 (lower panels, Figure 7).

DISCUSSION

Opioids remain the drugs of choice for treating moderate or severe pain, although their usefulness is limited by unwanted effects and by their ineffectiveness in some patients. Efforts continue toward improving the effectiveness of opioids, including combining them with other drugs. For example, indirect-acting 5-HT agonists (eg, SSRIs) are commonly coadministered with opioids for treating pain. Moreover, some drugs that are used to treat pain (eg, tramadol) have μ-opioid agonist actions and, like SSRIs, block the reuptake of 5-HT. It is unclear which of the many different 5-HT receptor subtypes contribute to the improved effectiveness of opioids when coadministered with drugs acting on 5-HT systems. The current study shows that indirect- and direct-acting 5-HT receptor agonists enhance the antinociceptive effects of morphine in rhesus monkeys. Moreover, this interaction has some selectivity insofar as the same 5-HT receptor agonists do not enhance, and in some cases attenuate, the discriminative stimulus effects of morphine. These results support the notion that when administered in combination with a 5-HT receptor agonist, smaller doses of opioids can be used to treat pain. The ability to use smaller doses of opioids, particularly for extended treatment, would likely result in fewer unwanted effects (eg, constipation, tolerance, and dependence).

It is well established that 5-HT systems have a role in nociception and that drugs acting on 5-HT systems can exert antinociceptive effects. For example, indirect-acting 5-HT receptor agonists can enhance the antinociceptive effects of opioids in rats (Larson and Takemori, 1977; Hynes *et al*, 1985), monkeys (Banks *et al*, 2010; Gatch *et al*, 1998), and humans (Coda *et al*, 1993). The results of this current study confirm the ability of indirect-acting 5-HT receptor agonists to enhance antinociceptive effects of opioids. For example, under conditions where fenfluramine alone had antinociceptive effects (50 °C water), fenfluramine increased the potency of morphine, shifting the dose-response curve leftward. Moreover, under conditions where fenfluramine was without effect (55 °C water), fenfluramine also shifted the morphine dose-response curve leftward. Enhancement of the antinociceptive effects of morphine by fenfluramine appears to involve 5-HT_{2A} receptors insofar as this enhancement was prevented by the 5-HT_{2A} receptor antagonist MDL100907. In contrast, the 5-HT_{1A} receptor antagonist WAY100635, at a dose that blocks behavioral effects of direct-acting 5-HT_{1A} receptor agonists (see, eg, Figure 2), failed to prevent enhancement of morphine antinociception by fenfluramine. Fenfluramine causes the release of 5-HT that acts on multiple receptor subtypes (McCreary *et al*, 2003), and interactions among those subtypes might be important for understanding the therapeutic effects of indirect-acting 5-HT receptor agonists, as suggested by recent studies examining combinations of drugs acting selectively on different 5-HT receptor subtypes (Li *et al*, 2010).

Drug discrimination procedures are pharmacologically selective and can be predictive of the abuse potential of drugs in humans. In the current study a discrimination procedure was used to examine whether 5-HT receptor agonists that enhance the antinociceptive effects of morphine also enhance other effects of morphine. Doses of fenfluramine smaller than those shifting the morphine antinociception dose-response curve to the left shifted the morphine discrimination dose-response curve to the right

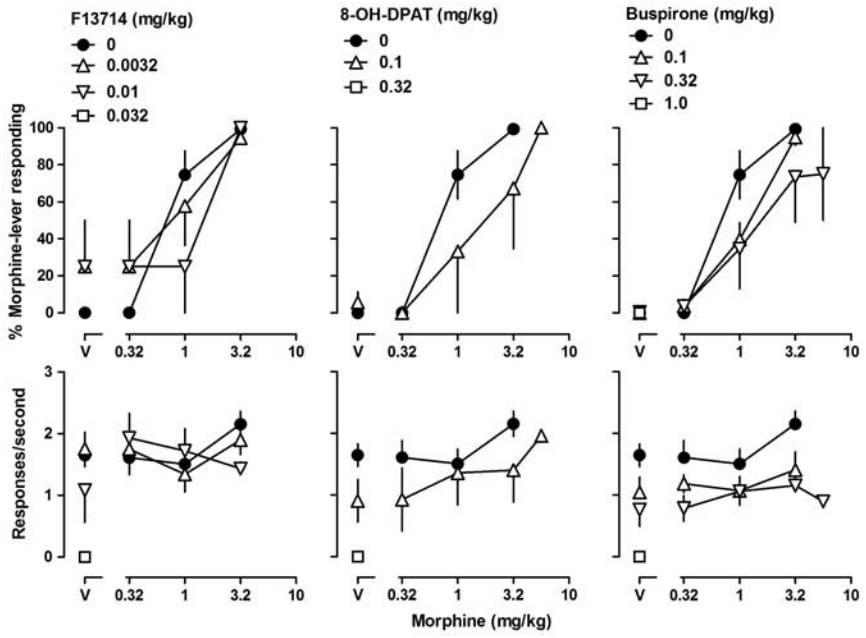

Figure 6 The discriminative stimulus effects of morphine alone and in combination with the 5-HT_{1A} receptor agonists F13714, 8-OH-DPAT, or buspirone. See Figure 5 for other details.

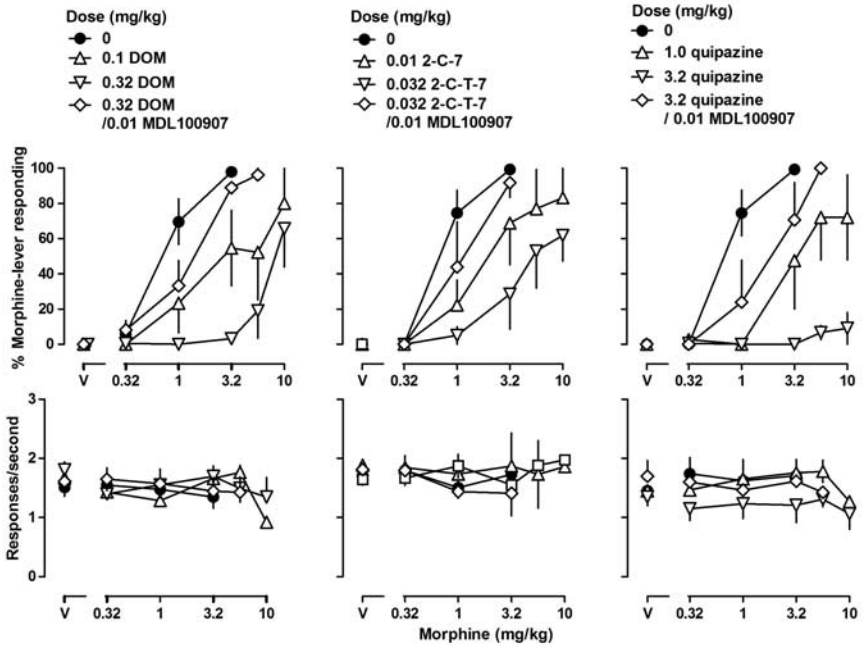

Figure 7 The discriminative stimulus effects of morphine alone, in combination with the 5-HT_{2A} receptor agonists DOM, 2C-T-7, or quipazine, and in combination with DOM, 2C-T-7, or quipazine as well as the 5-HT_{2A} receptor antagonist MDL100907. See Figure 5 for other details.

and down. In rats, dexfenfluramine, the active isomer of fenfluramine, did not modify the discriminative stimulus effects of morphine (Higgins *et al*, 1993). Differences in the effects of indirect-acting 5-HT receptor agonists on the morphine discriminative stimulus between the previous study and this study might be because of differences in the pharmacology of racemic fenfluramine and dexfenfluramine or because of differences in experimental details: schedules of reinforcement (FR 10 *vs* FR 5); reinforcers (food presentation *vs* stimulus shock termination); or species used (rat *vs* rhesus monkey). There is evidence, for example, indicating that interactions between 5-HT_{1A} and 5-HT_{2A} receptor agonists are qualitatively different between rats and monkeys (Li *et al*, 2010). Nevertheless, these results indicate that 5-HT receptor agonists do not enhance all effects of morphine, further supporting the view that combinations of 5-HT receptor agonists and opioids do not confer increased risk of abuse and dependence.

Direct-acting 5-HT_{1A} receptor agonists administered alone can exert antinociceptive effects on mice and rats (Crisp *et al*, 1991; Fasmer *et al*, 1986; Xu *et al*, 1994), and when administered in combination they can attenuate the antinociceptive effects of morphine (Millan and Colpaert, 1991). In monkeys, 5-HT_{1A} receptor agonists had antinociceptive effects, increasing tail-withdrawal latency from 50 °C but not 55 °C water. Moreover, pretreatment with a 5-HT_{1A} receptor agonist shifted the morphine dose-response curve leftward (for 50 and 55 °C water); similar effects were obtained with 5-HT_{1A} receptor agonists that are known to vary significantly in efficacy (see, eg, Koek *et al*, 2001). However, doses of 5-HT_{1A} receptor agonists that shifted the morphine dose-response curve leftward were the same doses that eliminated responding in monkeys discriminating morphine (ie, 0.032 mg/kg F13714, 0.32 mg/kg 8-OH-DPAT, and 1.0 mg/kg buspirone; Figures 2 and 6). Nevertheless, shifts leftward in the morphine dose-response curve by F13714, 8-OH-DPAT, and buspirone were prevented by the 5-HT_{1A} receptor antagonist, demonstrating the role of this receptor subtype in these drug interactions. Isobolographic presentation of the data indicate that the interaction between morphine and 5-HT_{1A} receptor agonists is additive. Possible differences in the interaction between opioids and 5-HT_{1A} receptor agonists between rats and non-human primates might reflect fundamental differences in the neurobiology of 5-HT systems across species (see, eg, Li *et al*, 2010).

When administered alone, 5-HT_{2A} receptor agonists also increased tail-withdrawal latency from 50 °C but not 55 °C water. Pretreatment with a 5-HT_{2A} receptor agonist shifted the morphine dose-response curve leftward (for 50 and 55 °C water). In contrast to 5-HT_{1A} receptor agonists, which had activity in the antinociception study only at doses that eliminated operant responding, 5-HT_{2A} receptor agonists shifted the morphine antinociception dose-response curve leftward at doses that had no effect on operant responding (Figures 2 and 7). Shifts leftward in the morphine dose-response curve by DOM, 2C-T-7, and quipazine were prevented by the 5-HT_{2A} receptor antagonist MDL100907, demonstrating the role of this receptor subtype in these drug interactions. Also, in contrast to the interaction between morphine and 5-HT_{1A} receptor agonists (ie, additivity), the interaction between morphine and 5-HT_{2A} receptor agonists appeared to be supra-additive. Thus, morphine was more potent to produce antinociception when administered together with a 5-HT_{2A} receptor agonist, even at doses of agonists that were without effect when given alone (ie, 55 °C water). Moreover, these agonists do not enhance all of the effects of morphine. In fact, the same doses of 5-HT_{2A} receptor agonists that enhanced the antinociceptive effects of morphine markedly attenuated the discriminative stimulus effects of morphine; both enhancement of antinociceptive effects and attenuation of discriminative stimulus effects were prevented by 0.01 mg/kg of the 5-HT_{2A} receptor antagonist MDL100907. Although 5-HT_{2A} agonists appear to selectively enhance antinociceptive effects of morphine, some 5-HT_{2A} receptor agonists have other behavioral effects that might contraindicate their use for treating pain (hallucinations).

In summary, both indirect-acting (5-HT releaser) and direct-acting (5-$HT_{!A}$ and 5-HT_{2A} receptor agonists) 5-HT receptor agonists increased the potency of morphine in an assay of antinociception, while not increasing, and in some cases decreasing, the potency and/or effectiveness of morphine in an drug discrimination assay. Fenfluramine and 5-HT_{2A} receptor agonists increased the antinociceptive effects of morphine at doses that did not affect operant responding where 5-HT_{1A} receptor agonists increased the potency of morphine only at doses that eliminated operant responding. Thus, indirect-acting 5-HT receptor agonists and direct-acting 5-HT_{2A} receptor agonists might have a comparatively larger margin of safety with regard to their possible combined use with opioids for treating pain. It is possible, for example, that small doses of direct-acting 5-HT receptor agonists in combination with small doses of opioids could provide effective pain treatment and that long-term use of smaller doses would yield fewer adverse effects (eg, constipation, tolerance, and dependence). Although more work is needed to address whether the drug combination has higher abuse liability than opioids alone, to the extent that the discriminative stimulus effects of morphine parallel the subjective effects of opioids in humans (Schuster and Johanson, 1988), this study also suggests that these drug combinations might not increase the abuse liability of opioids.

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DISCLOSURE

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