Published in final edited form as:

Psychopharmacology (Berl). 2013 February ; 225(4): 791–801. doi:10.1007/s00213-012-2870-2.

Modification of the behavioral effects of morphine in rats by serotonin (5-HT)_{1A} and 5-HT₂ receptor agonists: antinociception, drug discrimination, and locomotor activity

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Abstract

Rationale—Indirect-acting serotonin (5-HT) receptor agonists can enhance the antinociceptive effects of morphine; however, the specific 5-HT receptor subtype(s) mediating this enhancement is not established.

Objective—This study examined interactions between morphine and both 5-HT_{1A} and 5-HT_{2A} receptor agonists in rats using measures of antinociception (radiant heat tail flick and warm water tail withdrawal), drug discrimination (3.2 mg/kg morphine versus saline), and locomotion.

Methods—Male Sprague-Dawley rats (n=7–8 per group) were used to examine the effects of morphine alone and in combination with DOM (5-HT_{2A} agonist) and 8-OH-DPAT (5-HT_{1A} agonist).

Results—DOM did not modify antinociceptive or discriminative stimulus effects while modestly attenuating locomotor-stimulating effects of morphine; the effect of DOM (0.32 mg/kg) on morphine-induced locomotion was prevented by the 5-HT_{2A} receptor selective antagonist MDL 100907. In contrast, 8-OH-DPAT (0.032–0.32 mg/kg) fully attenuated the antinociceptive effects (both procedures), did not modify the discriminative stimulus effects, and enhanced (0.32 mg/kg) the locomotor-stimulating effects of morphine. These effects of 8-OH-DPAT were prevented by the 5-HT_{1A} receptor selective antagonist WAY100635.

Conclusion—Agonists acting at 5-HT_{1A} or 5-HT_{2A} receptors do not modify all effects of mu opioid receptor agonists in a similar manner. Moreover, interactions between 5-HT and opioid receptor agonists vary significantly between rats and nonhuman primates, underscoring the value of comparing drug interactions across a broad range of conditions and in multiple species.

Introduction

Serotonergic (5-HT) systems are involved in the transmission of nociception at both the spinal and supraspinal levels and increasing 5-HT neuronal activity can produce antinociception independently or enhance antinociceptive effects of other drugs. For example, the 5-HT releaser fenfluramine has antinociceptive activity in animal models that mimic both acute and neuropathic pain conditions (Rochat et al., 1982; Wang et al., 1999)

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and selective 5-HT reuptake inhibitors (SSRIs) have antinociceptive activity in several models of acute pain in rodents (Schreiber et al., 1996; Singh et al., 2001; Duman et al., 2004; Duman et al., 2006).

SSRIs as well as non-selective reuptake inhibitors (e.g., duloxetine which blocks the reuptake of 5-HT and norepinephrine; Smith and Nicholson, 2007) are commonly used to treat pain, often in combination with other drugs; however, the role of specific 5-HT receptor subtypes in mediating the antinociceptive (or antinociceptive enhancing) effects of SSRIs is not well established. For example, intrathecal administration of 5-HT produces antinociceptive effects that appear to be mediated by 5-HT₂ receptor activation (Crisp et al., 1991); however, antinociceptive effects of the SSRI paroxetine are enhanced by 5-HT₂ receptor blockade (Kesim et al., 2005). These seemingly inconsistent findings are likely due, in part, to the complex neuropharmacology of SSRIs (i.e., blocking reuptake of 5-HT that can act at many different receptors) and might be clarified by examining the effects of drugs acting selectively and directly at 5-HT receptor subtypes. For example, both central microinjection and intrathecal administration of 5-HT₁ receptor agonists, but not 5-HT₂ receptor agonists, produces antinociceptive effects in a tail flick procedure in rats (Xu et al., 1994; Mamade et al., 1997), implicating a role for 5-HT₁ but not 5-HT₂ receptors in mediating antinociception.

Several studies have demonstrated that increasing the concentration of 5-HT enhances the antinociceptive effects of mu opioid receptor agonists. For example, administration of the 5-HT precursor 5-hydroxytryptophan (5-HTP) or an SSRI (fluoxetine, citalopram, or paroxetine) increases morphine antinociception in mice and rats, respectively (Dewey et al., 1970; Larson and Takemori, 1977; Hynes et al., 1985; Lee et al., 2012). Moreover, fluoxetine and the 5-HT releaser fenfluramine enhance the antinociceptive effects morphine in nonhuman primates (Gatch et al., 1998; Li et al., 2011). Similarly, in humans fluoxetine and fenfluramine enhance morphine analgesia (Coda et al., 1993; Erjavec et al., 2000). The extent to which direct-acting 5-HT receptor agonists modify the antinociceptive effects of opioid receptor agonists is not fully established, although 5-HT_{1A} receptor agonists inhibit the antinociceptive effects of morphine in mice and rats (Berge et al., 1985; Millan and Colpaert, 1990; Millan and Colpaert, 1991).

Although relatively few direct comparisons are available, there is evidence suggesting that the interaction between 5-HT receptor (e.g., 5-HT_{1A}) agonists and opioid receptor agonists might vary significantly among species. For example, in contrast to some results obtained in rodents, 5-HT_{1A} receptor agonists do not inhibit, but rather modestly enhance, the antinociceptive effects of morphine in nonhuman primates (Li et al., 2011). Moreover, in nonhuman primates 5-HT_{2A} receptor agonists markedly enhance the antinociceptive effects of morphine and this interaction is greater-than-additive (Li et al., 2011). These findings, together with other studies in which the effects of drugs acting on 5-HT systems, particularly when studied in combination, were significantly different between rodents and nonhuman primates (Li et al., 2010), raising the possibility that interactions between 5-HT receptor agonists and opioid receptor agonists might also vary among species.

In light of recently published data from this laboratory (Li et al., 2011) showing significant interactions between morphine and 5-HT receptor agonists in nonhuman primates, the current study examined the effects of a 5-HT_{1A} receptor agonist and a 5-HT_{2A} receptor agonist on the antinociceptive, discriminative stimulus, and locomotor-stimulating effects of morphine in rats. The goal of this study was to test the generality of interactions between 5-HT and opioid receptor agonists across different behavioral effects in rats and also to determine whether interactions (i.e., between 5-HT receptor agonists and opioid receptor

agonists) observed in rats are consistent with results obtained with the same drugs in other species (e.g., rhesus monkeys).

Materials and methods

Subjects

Thirty-eight adult male Sprague-Dawley rats (Harlan, Indianapolis, IN) were housed individually on a 12/12-h light/dark cycle (behavioral experiments were conducted during the light period) with free access to water in the home cage. Sixteen rats were used in the antinociception study (8 for each of the two procedures), 8 rats in the drug discrimination study, and 14 rats in the locomotor activity study. Body weight of rats in the drug discrimination study was maintained at 350 g by adjusting the amount of rodent chow (Rodent sterilizable diet; Harlan Teklad, Madison, WI) that was provided in the home cage after daily sessions. All other rats had free access to food in the home cage. Animals were maintained and experiments were conducted in accordance with the Institutional Animal Care and Use Committee, The University of Texas Health Science Center at San Antonio, and with the *2011 Guide for the Care and Use of Laboratory Animals*, Eighth Edition (Institute of Laboratory Animal Research, Division on Earth and Life Studies, National Research Council of the National Academy of Sciences, Washington DC).

Apparatus

Drug discrimination studies were conducted with commercially available chambers (model ENV-008CT; MED Associates Inc., St. Albans, VT) located within sound-attenuating, ventilated cubicles (model ENV-022M; MED Associates Inc.). The chambers were equipped with two levers, associated stimulus lights, and a food hopper. Data were collected with MED-PC IV software (MED Associates Inc.), an interface, and a computer.

Locomotor activity studies were conducted with Lexan® polycarbonate (General Electric Structured Products, Mt. Vernon, IN, USA) chambers (26 × 61 × 23 cm high; manufactured by Instrumentation Services, University of Texas Health Science Center, San Antonio, TX) equipped with metal grid floors and located in sound-attenuating, ventilated cubicles (MED Associates Inc., St. Albans, Vermont, USA). Horizontal activity (locomotor activity) was measured with 4 pairs of infrared photo beams (Multi-Varimex, Columbus Instruments, Columbus, Ohio, USA) positioned 4 cm above the floor of the chamber. The photo beams were separated by 15 cm with two of the photo beams located 8 cm from the ends of the chamber.

Procedure

Antinociception—Two antinociception procedures were used in this study. For the radiant heat tail flick test, a commercially available apparatus (Columbus Instruments, Columbus, OH) delivered radiant heat to the middle 1/3 of the tail; the latency for rats to remove their tail from the heat source was measured with an automatic timer (resolution=1/100 s). The intensity of the radiant heat stimulus was adjusted for individual rats so that the baseline (i.e., no drug) tail flick latency was 3–4 s. When a rat did not flick its tail within 8 s (i.e., maximum latency cut off) during a test, the stimulus was terminated and a latency of 8 s was recorded. The inter-injection interval was 30 min with tail flick latencies measured beginning 28 min after each injection.

For the warm water tail withdrawal procedure (Li et al., 2007; Thorn et al., 2011), rats were lightly restrained and the distal 5–10 cm of the tail was immersed in a thermal flask containing 40, 50, or 55° C water (only data for 50° C are reported). The order for testing the 3 different temperatures varied nonsystematically among rats and across cycles. When a

rat did not remove its tail from the thermos within 20 s (i.e. maximum latency cut off) during a test, the experimenter removed the tail from the water and a latency of 20 s was recorded. Test sessions began with control (no drug) determinations for each temperature. Every 30 min (i.e., one cycle), tail withdrawal latencies were measured for all three temperatures with 1 min between determinations. The latency was recorded with a hand-operated stopwatch. Rats in the radiant heat tail flick and the warm water tail withdrawal studies were tested not more than once per week.

For both antinociception procedures, prior to drug testing at least two control sessions were conducted with repeated injections of saline in order for rats to adapt to handling and to the procedure. A multiple-cycle cumulative-dosing procedure was used to determine dose-response curves for morphine administered alone and morphine administered in rats that had received an acute injection of DOM (maximum of 5 injections per session). Because 8-hydroxy-*N,N*-dipropyl-2-aminotetralin (8-OH-DPAT) has a relatively short duration of action in rats (Kleven and Koek, 1998), it was not administered as a pretreatment to a morphine dose-response determination. Instead, a dose of morphine that significantly increased tail flick (radiant heat) and tail withdrawal (warm water) latencies (10.0 mg/kg) was administered first, followed by administration of increasing doses of 8-OH-DPAT. Because 8-OH-DPAT attenuated the antinociceptive effects of morphine, an additional experiment tested whether the 5-HT_{1A} receptor selective antagonist WAY100635 prevented this effect of 8-OH-DPAT. Thus, for each procedure each rat received three dose-response determinations with morphine (alone and with two different doses of DOM) and two tests with a single injection of 10.0 mg/kg morphine (with increasing doses of 8-OH-DPAT alone and after pretreatment with WAY100635).

Drug discrimination—Rats were trained to discriminate between injections of saline and 3.2 mg/kg morphine while responding under a fixed ratio (FR) 5 schedule of food presentation as described previously (Li et al., 2009). Daily sessions comprised 2–6 cycles and each cycle began with a 10-min timeout period, during which the chamber was dark and lever presses had no programmed consequence, followed by a 5-min response period, during which stimulus lights above both levers were illuminated and the FR 5 schedule of food presentation was active on one (training sessions) or both (test sessions) levers. Five consecutive responses on the left lever delivered a food pellet in saline training cycles and 5 consecutive responses on the right lever delivered a food pellet in morphine training cycles. A maximum of one injection of morphine was administered in a training session (some sessions included only saline cycles) and that cycle was followed by a single sham injection cycle when only responding on the morphine-associated lever delivered a food pellet.

Rats were considered to be under adequate stimulus control for testing when the following criteria were satisfied for 5 consecutive or 6 of 7 consecutive sessions: at least 90% of the total responses were made on the correct lever and fewer than 5 responses (one FR) were made on the incorrect lever before delivery of the first food pellet. Thereafter, tests were conducted whenever the same criteria were satisfied for two consecutive sessions, one saline training session and one morphine training session. Test sessions were identical to training sessions except that 5 consecutive responses on either lever delivered food and different doses of drugs (alone or in combination) were administered. Drugs were studied up to doses that occasioned at least 90% responding on the morphine-associated lever or to doses that markedly decreased the rate of responding.

Locomotor activity—Prior to testing, all rats were habituated to the test chamber for one hour per day on 3 different days. Next, locomotion was assessed in a session when saline injections were given at 0, 30, 50, 70, 90 and 110 min (6 injections per session). Similar saline test sessions were also conducted during and after studies with morphine and 5-HT

receptor agonists. Locomotion was continuously recorded with locomotor activity measured and recorded every 5 min for 130 min. For morphine dose-response determinations, different doses of morphine (0.32–17.8 mg/kg) were administered at 30, 50, 70, 90 and 110 min using a cumulative-dosing procedure. For drug combination studies, a 5-HT receptor agonist (or agonist with antagonist) was administered at the 30 min time point together with a dose of 0.32 mg/kg morphine. One group of 7 rats was used to examine the effects of 8-OH-DPAT in combination with morphine and another group of 7 rats was used to examine the effects of DOM in combination with morphine. Rats in the locomotor activity study were tested no more often than once per week. During this 3-month study, each rat was tested on 11 occasions as follows: 3 habituation sessions; 3 saline test sessions; and 5 drug test sessions.

Drugs

The compounds used in this study were the following: morphine sulfate and 1-(2,5-dimethoxy-4-methylphenyl)-2-aminopropane hydrochloride (DOM), both from the Research Technology Branch, National Institute on Drug Abuse, Rockville, MD); (±)2,3-dimethoxyphenyl-1-[2-(4-piperidine)-methanol] hydrochloride (MDL100907) was synthesized by Kenner Rice (Ullrich and Rice, 2000); 8-hydroxy-2-(di-n-propylamino) tetralin hydrochloride (8-OH-DPAT) was purchased from Sigma-Aldrich (St. Louis, MO); *N*-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-*N*-(2-pyridinyl)cyclohexanecarboxamide dihydrochloride (WAY100635) was a gift from Dr. Adrian Newman-Tancredi (Centre de Recherche Pierre Fabre, Castres, France). Compounds were dissolved in saline with the exception of MDL100907 which was dissolved in 20% dimethylsulfoxide (v/v). Doses are expressed as the form of the drug listed above in mg per kg of body weight. All drugs were administered intraperitoneally and the injection volumes were 0.2–1.0 ml.

Data analyses

For the antinociception study, tail withdrawal latency was expressed as a percentage of the maximal possible effect using the following formula: $(\text{test latency} - \text{control latency} / \text{cutoff} - \text{control latency}) \times 100$, where the control latency was defined as the latency determined in the absence of drug. The maximum possible effect was calculated for each individual and then averaged to obtain a group mean. Potencies were obtained by estimating the dose required to produce 50% of the maximum possible effect (ED_{50}) using linear regression, along with 95% confidence limits (95% CL). For the effects of 8-OH-DPAT on morphine antinociception, one way repeated measures ANOVA followed by Dunnett's post hoc analysis was used. For all other drug interaction studies a two-way repeated measures ANOVA was used followed by Bonferroni post hoc analysis, with dose of morphine or 8-OH-DPAT serving as a within-subjects factor and DOM or WAY100635 pretreatment as a between-subjects factor.

For the drug discrimination study, the following two dependent variables were measured: the percentage of responses on the drug-appropriate lever during the response period, calculated by dividing the number of responses on the drug-appropriate lever by the total number of responses on both levers, and multiplying by 100; and the rate of responding during the response period, calculated by dividing the total number of responses made on both levers by the duration of the response period in seconds (excluding timeouts). 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 obtained by estimating the dose required to generate 50% responding on the drug-appropriate lever (ED_{50}), along with 95% confidence limits (95% CL), using linear regression.

For the locomotor activity study, activity counts were recorded continuously; data from the first 30 min were discarded because locomotion during the first 30 min after rats were placed in the chamber was more variable and higher than locomotor activity during the remainder of the session. Data were collected in four 5-min bins across each 20-min cycle. Data from the first 5-min bin (immediately after injection) were not used since the handling and the injection procedure itself often stimulated locomotion for several minutes. Thus, for each of the 5 cycles comprising a daily session, only data from the last 3 bins (15 min) were used for analyses. Locomotor activity data were expressed as a percentage of the average of three saline-injection (control) sessions that were determined at the beginning, middle, and end of this study, with each rat serving as its own control. Drug interaction data were analyzed with two-way repeated measures ANOVA followed by Bonferroni post hoc analysis, with dose of morphine as a within-subjects factor and drug pretreatment as a between-subjects factor.

Results

Antinociception

Under control conditions the average latency for rats to remove their tails (mean $\pm$ SEM) from the radiant heat was 3.64 ± 0.11 sec. Morphine dose-dependently increased the latency for rats to remove their tails from radiant heat (circles, Fig 1; ED_{50} [95% CL] = 4.81 [3.99 , 5.80]) with greater than 90% of the maximum possible effect observed after administration of 10.0 mg/kg. When administered alone, a dose of 1.0 mg/kg DOM had no effect on tail flick latency whereas a dose of 3.2 mg/kg DOM significantly increased tail flick latency (open symbols above “V”, Fig 1). Pretreatment with either of these doses of DOM did not significantly alter the morphine dose-response curve for tail flick latency, with the exception that the effect of 1.0 mg/kg of morphine was increased in rats that had been pretreated with 3.2 mg/kg DOM. A two-way repeated measures ANOVA revealed a significant main effect for DOM pretreatment ($F[2, 63] = 6.85$, $P < 0.05$). Post hoc analysis revealed that 3.2 mg/kg DOM alone significantly increased tail flick latency ($P < 0.01$) and that a combination of 3.2 mg/kg DOM and 1.0 mg/kg morphine had a greater effect than this dose of morphine alone ($P < 0.05$).

Under control conditions the average latency for rats to remove their tails (mean $\pm$ SEM, in seconds) from 40°, 50°, and 55° C water was 20 ± 0 (i.e., the maximal possible effect), 6.65 ± 0.21 , and 3.39 ± 0.17 sec, respectively. Because the doses of morphine administered produced less than 50% of the maximum possible effect with 55°C water, the data from 55°C water are not presented. Morphine dose-dependently increased the latency for rats to remove their tails from 50° C water (circles, left panel, Fig 2; $ED_{50} = 4.01$ [3.13 , 5.14]) with greater than 90% of the maximum possible effect observed after administration of 10.0 mg/kg. DOM alone did not increase tail withdrawal latency; however, pretreatment with 3.2 mg/kg of DOM increased the effect obtained with 1.0 mg/kg morphine. Otherwise the morphine dose-response curve for warm water tail withdrawal latency was not affected by DOM (left panel, Fig 2). A two-way repeated measures ANOVA revealed no significant main effect for DOM pretreatment ($F[2, 63] = 0.99$, $P > 0.05$). Post hoc analysis revealed that 3.2 mg/kg DOM significantly increased the effects of 1.0 mg/kg morphine ($P < 0.05$).

Acute administration of 10.0 mg/kg of morphine produced 71% of the maximum possible effect in the radiant heat procedure (square above “V”, left panel, Fig 3) and this effect of morphine was dose-dependently attenuated by 8-OH-DPAT. A one-way repeated measures ANOVA revealed that the effect of 8-OH-DPAT in combination with morphine was significantly different from morphine alone ($F[4, 39] = 35.59$, $P < 0.0001$). Post hoc analysis indicated that doses of 0.032, 0.1, and 0.32 mg/kg of 8-OH-DPAT significantly attenuated the effect of 10.0 mg/kg morphine. Attenuation of the effect of morphine on tail flick latency

by 8-OH-DPAT was prevented by pretreatment with WAY 100635 (diamonds, left panel, Fig 3). A two-way repeated measures ANOVA revealed a significant main effect for WAY100635 ($F[4, 56] = 49.21, P < 0.0001$).

Acute injection with 10.0 mg/kg morphine produced 100% of the maximum possible effect in the warm water tail withdrawal procedure (square above "V", right panel, Fig 3) and this effect of morphine was dose-dependently attenuated by 8-OH-DPAT. A one-way repeated measures ANOVA revealed that the effect of 8-OH-DPAT in combination with morphine was significantly different from morphine alone ($F[4, 39] = 95.15, P < 0.0001$). Post hoc analysis showed that 8-OH-DPAT dose-dependently attenuated the effect of morphine with all doses of 8-OH-DPAT significantly decreasing the effect of morphine. Attenuation of the effect of morphine on tail withdrawal latency by 8-OH-DPAT was prevented by pretreatment with WAY 100635 (diamonds, right panel, Fig 3). A two-way repeated measures ANOVA revealed a significant main effect for WAY100635 ($F[4, 56] = 17.65, P < 0.0001$).

Drug discrimination

Morphine increased responding on the drug-associated lever in a dose-related manner with doses of 3.2 mg/kg and 5.6 mg/kg occasioning more than 80% drug-lever responding ($ED_{50} = 1.52 [0.96, 2.40]$; circles, left panels, Fig 4). Acute administration of 0.1 (data not shown) or 0.32 mg/kg of DOM occasioned responding predominantly on the saline-associated lever (data point above "V", upper left panel, Fig 4) without markedly affecting response rate (data not shown). Pretreatment with the same doses of DOM did not significantly modify the discriminative stimulus effects of morphine (0.1 mg/kg DOM, morphine $ED_{50} = 1.01 [0.52, 1.93]$; 0.32 mg/kg DOM, morphine $ED_{50} = 1.73 [1.67, 1.80]$). A larger dose of DOM (1.0 mg/kg) alone significantly decreased the rate of responding (data not shown) and was not studied in combination with morphine.

Acute administration of 0.032 (data not shown) or 0.1 mg/kg 8-OH-DPAT occasioned responding predominantly on the saline-associated lever (data point above "V", lower left panel, Fig 4) without markedly affecting response rate (data not shown). Pretreatment with the same doses of 8-OH-DPAT did not significantly modify the discriminative stimulus effects of morphine (0.032 mg/kg 8-OH-DPAT, morphine $ED_{50} = 1.08 [0.60, 1.94]$; 0.1 mg/kg 8-OH-DPAT, morphine $ED_{50} = 1.89 [1.05, 3.39]$). A larger dose of 8-OH-DPAT (0.32 mg/kg) alone eliminated responding (data not shown) and was not studied in combination with morphine.

Locomotor activity

Morphine dose-dependently increased locomotor activity in both groups of rats with the largest increases (i.e., to more than 300% of control) observed after the administration of 10.0 mg/kg. A larger dose of morphine occasioned comparatively less locomotion, resulting in an inverted U-shaped dose-response curve (open circles, top panels, Fig 5). The morphine dose-response curves for locomotion were similar between the two groups of rats (compare circles in top panels, Fig 5) and they did not change markedly from the beginning to the end of this study (compare open and closed circles within each upper panel, Fig 5).

Pretreatment with DOM at doses (0.1 and 0.32 mg/kg) that alone did not affect locomotor activity (data not shown), only decreased morphine-stimulated locomotion (middle left panel, Fig 5). A two-way repeated measures ANOVA indicated a significant main effect of DOM ($F[2, 72] = 5.46, P < 0.05$). Post hoc analysis revealed that 0.32 mg/kg DOM significantly decreased the effect of 10.0 mg/kg morphine ($P < 0.01$). The decrease in morphine-induced locomotion by DOM was prevented by MDL100907 (lower right panel,

Fig 5). A two-way repeated measures ANOVA showed a significant main effect of MDL100907 ($F[2, 72] = 8.06, P < 0.005$). Post hoc analysis revealed that suppression by DOM of the effects of 10.0 ($P < 0.05$) and 17.8 mg/kg ($P < 0.05$) morphine was significantly prevented by 0.01 mg/kg MDL100907 (lower left panel, Fig 5).

Pretreatment with a dose of 8-OH-DPAT (0.32 mg/kg) that alone did not modify locomotor activity (data not shown), significantly enhanced morphine-induced locomotion (inverted triangles, middle right panel, Fig 5). A two-way repeated measures ANOVA indicated a significant main effect of 8-OH-DPAT ($F[2, 72] = 6.67, P < 0.005$). Post hoc analysis revealed that 0.32 mg/kg 8-OH-DPAT significantly increased the locomotion observed after the administration of 10.0 ($P < 0.01$) or 17.8 mg/kg ($P < 0.001$) morphine. Enhancement of morphine-induced locomotion by 8-OH-DPAT was prevented by WAY100635 (lower right panel, Fig 5). A two-way repeated measures ANOVA showed a significant main effect of WAY100635 ($F[2, 72] = 6.40, P < 0.05$). Post hoc analysis revealed that enhancement of the locomotor-stimulating effect of 17.8 mg/kg morphine by 0.32 mg/kg 8-OH-DPAT was prevented by 0.1 mg/kg WAY100635. A smaller dose of 8-OH-DPAT (0.1 mg/kg) did not significantly affect morphine-induced locomotion (triangles, middle right panel, Fig 5).

Discussion

The role of 5-HT in pain is well established although the specific mechanisms by which drugs acting on 5-HT systems modify nociception (directly or by altering the actions of other drugs) is not fully understood. It is clear that global elevation in 5-HT, by increasing 5-HT synthesis (e.g., 5-HT precursor 5-HTP), increasing 5-HT release (e.g., fenfluramine), or decreasing 5-HT reuptake (e.g., SSRIs), can result in antinociception (Liang et al., 2004; RoCHAT et al., 1982; Wang et al., 1999; Schreiber et al., 1996; Singh et al., 2001; Duman et al., 2004; Duman et al., 2006). However, such a global increase in 5-HT results in a variety of neuropharmacological actions mediated by as many as 14 different 5-HT receptor subtypes that have been identified. The complexity of such a non-selective enhancement of 5-HT signaling precludes a clear understanding of which specific receptor subtype(s) mediates the antinociceptive effects of indirect-acting 5-HT receptor agonists.

Similarly, the role of specific 5-HT receptor subtypes in modulating (enhancing or attenuating) antinociceptive effects of other drugs (e.g., μ opioid receptor agonists) is unclear. Converging lines of evidence indicate that 5-HT receptor activation in brain is necessary to enhance the antinociceptive effects of opioid receptor agonists although little is known regarding which 5-HT receptor(s) contributes to this interaction (Dewey et al., 1970; Larson and Takemori, 1977; Hynes et al., 1985; Gatch et al., 1998). Activation of 5-HT_{1A} receptors attenuates the antinociceptive effects of morphine in rodents (Berge et al., 1985; Millan and Colpaert, 1990; Millan and Colpaert, 1991) whereas activation of 5-HT_{1A} receptors enhances the antinociceptive effects of morphine in nonhuman primates (Li et al., 2011). Moreover, in nonhuman primates, 5-HT₂ receptor agonists also markedly enhance the antinociceptive effects of morphine (Li et al., 2009). However, there are few systematic comparisons, and even fewer cross species comparisons, of interactions between 5-HT and opioid receptor agonists.

One goal of the current study was to examine whether 5-HT receptor activation enhances antinociceptive effects and attenuates discriminative stimulus effects of morphine in rats, as it does in nonhuman primates. In contrast to results obtained in nonhuman primates, the 5-HT_{2A} receptor agonist DOM enhanced the antinociceptive effects of a small dose of morphine but only at doses of DOM that had modest antinociceptive activity alone. A similar effect was observed with DOM and morphine in both the radiant heat tail flick and the warm water tail withdrawal procedures; the morphine antinociception dose-response

curve was not shifted leftward by DOM in rats, as it is in nonhuman primates. Comparing data from rats (current study) and monkeys (Li et al., 2011), Fig 2 illustrates significant enhancement by DOM of the antinociceptive effects of morphine in monkeys but not in rats.

In contrast to results obtained with DOM in combination with morphine, the 5-HT_{1A} receptor agonist 8-OH-DPAT attenuated the antinociceptive effects of morphine in both procedures in rats, and this attenuation was prevented by the selective 5-HT_{1A} receptor antagonist WAY100635. Attenuation of the antinociceptive effects of morphine by a 5-HT_{1A} receptor agonist is consistent with other results obtained with rodents (Berge et al., 1985; Millan and Colpaert, 1990; Millan and Colpaert, 1991) and stand in contrast to results (i.e., enhancement) obtained with nonhuman primates (Li et al., 2011). The relatively small dose of 8-OH-DPAT (0.032 mg/kg) that attenuated the antinociceptive effects of morphine in this study might selectively activate presynaptic 5-HT_{1A} receptors, thereby decreasing 5-HT neurotransmission (Casanovas et al., 1997); decreased 5-HT neurotransmission has been shown to attenuate some effects of morphine (Berge et al., 1983). Although WAY100635 has affinity for dopamine receptors (Chemel et al., 2006), 8-OH-DPAT is highly selective for 5-HT_{1A} receptors; moreover, the doses of WAY100635 that prevented the effects of 8-OH-DPAT in the current study are the same as those shown to antagonize 5-HT_{1A} receptor mediated effects in other studies (e.g., Koek et al., 2000).

Data from the current study and from previous studies (e.g., Li et al., 2010) demonstrate that some effects of some drugs acting on 5-HT systems vary significantly between rodents and nonhuman primates, particularly with regard to drug interactions. However, similar to what is observed in nonhuman primates, 8-OH-DPAT did not significantly modify the discriminative stimulus effects of morphine up to doses of 8-OH-DPAT that disrupted responding; a dose of 0.1 mg/kg 8-OH-DPAT shifted the morphine discrimination dose-response curve slightly but not significantly to the right in monkeys (Li et al., 2011) and had no marked effect on the morphine dose-response curve in rats (compare lower panels, Fig 4). In rats, DOM also failed to alter the morphine discrimination dose-response curve up to the largest dose (0.32 mg/kg) that could be studied; however, that dose of DOM shifted the morphine discrimination dose-response curve more than 10-fold to the right in monkeys (Li et al., 2011; compare upper panels, Fig 4). It is not likely that procedural differences across studies account for these discrepancies because a warm water tail withdrawal procedure was used (the same stimulus intensity [50° C] and the same drug [morphine]) in both rats and nonhuman primates. That the same interactions were observed with the warm water tail withdrawal in rats and with the radiant heat tail flick procedure in rats suggests that any small differences in procedure are not likely to account for qualitative differences in outcome. Similarly, a two-lever drug (morphine) versus vehicle discrimination procedure was used in rats and in monkeys and there is a considerable literature showing that the discriminative stimulus effects of morphine are qualitatively similar across species. Thus, it appears as though factors other than procedural ones must account for observed differences between species. The neurobiology of 5-HT systems varies among species; for example, the distribution and number of 5-HT_{1A} and 5-HT_{2A} receptors varies markedly between rodents and nonhuman primates (López-Giménez et al., 2001; Lucaites et al., 2005), although little is known about the functional consequences of these differences. One study systematically compared interactions between 5-HT_{1A} receptor agonists and 5-HT_{2A} receptor agonists in rats and nonhuman primates and found qualitative differences between the two species with 5-HT_{1A} receptor agonists attenuating the discriminative stimulus effects of DOM in nonhuman primates but not in rats (Li et al., 2010). Thus, significant differences in the interaction between 5-HT receptor agonists and opioid receptor agonists, comparing rats and nonhuman primates, in studies of antinociception as well as drug discrimination might result from differences in the underlying neurobiology of 5-HT systems across species.

One widely studied effect of morphine in rats is the induction of locomotion whereby progressively increasing doses stimulate then attenuate locomotion, resulting in an inverted U-shaped dose-response curve. It is thought that morphine stimulates locomotion by indirectly promoting the release of dopamine through disinhibition of inhibitory GABA interneurons (Spanagel, 1995). Antagonists acting at dopamine D₁ and D₂ receptors attenuate morphine induced locomotion as does the opioid receptor antagonist naloxone (Zarrindast and Zarghi, 1992). Similarly, the indirect-acting dopamine receptor agonist cocaine stimulates locomotion by preventing the reuptake and possibly promoting the release of dopamine (Woolverton and Johnson, 1992). The locomotor stimulating effects of cocaine are increased by 5-HT_{1A} receptor agonists, presumably by further increases in dopamine concentration (Carey et al., 2001; Carey et al., 2002; Muller et al., 2003; Nakamura et al., 2006). It is possible, therefore, that 8-OH-DPAT enhances morphine-induced locomotion by indirectly increasing dopamine concentration. The interaction between 5-HT₂ receptors and dopamine systems appears to be more complicated. Depending on the experimental condition, 5-HT_{2A} receptor activation can either increase or decrease dopamine release (Alex and Pehek, 2007). Moreover, 5-HT_{2A} and 5-HT_{2C} receptors have opposing effects in modulating dopamine release (Alex and Pehek, 2007). Although DOM has similar affinity at 5-HT_{2A} and 5-HT_{2C} receptors, the effects of DOM for attenuating morphine-induced locomotor-stimulation are likely mediated by 5-HT_{2A} receptors since those effects are prevented by the selective 5-HT_{2A} receptor antagonist MDL100907.

It is well established that 5-HT systems play a role in nociception and drugs acting on 5-HT systems (SSRIs) are commonly used together with other drugs to treat pain. However, by blocking the reuptake of neurotransmitter, SSRIs globally increase 5-HT which can act on a variety of 5-HT receptor subtypes. The current study, along with previously published studies, demonstrates a differential role for 5-HT receptor subtypes in modulating the antinociceptive effects of a mu opioid receptor agonist – the most widely used class of drugs for treating moderate-severe pain. The current study also demonstrates that not all effects of a mu opioid receptor agonist are similarly modulated by the same 5-HT receptor subtype selective agonist, with the 5-HT_{1A} receptor agonist 8-OH-DPAT attenuating morphine-induced antinociception, enhancing morphine-induced locomotion, and not affecting the discriminative stimulus effects of morphine. Finally, these results contribute to a growing literature showing that the effects of some drugs, administered alone but especially when administered in combination, are quantitatively and qualitatively different across some species.

Acknowledgments

This study was supported, in part, by grants R01DA05018 and K0517918 (Senior Scientist Award to CPF). A portion of this work was supported by the Intramural Research Programs of National Institute on Drug Abuse and National Institute on Alcohol Abuse and Alcoholism (KCR). The authors would like to thank Christopher Cruz, Margarita Gardea and Sonia Cano for their expert technical support.

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Fig. 1.

Antinociceptive effects of morphine alone and in combination with DOM (1.0 and 3.2 mg/kg) in a radiant heat tail flick procedure. Abscissa: dose of morphine in milligrams per kilogram body weight; ordinate: average ($\pm$ SEM) tail flick latency from radiant heat expressed as a percentage of the maximal possible effect (8 seconds). Points above "V" represent the effects of saline (circles) or DOM (triangles and squares) alone. $*=P<0.05$ compared to "V" or to the effects of morphine in the absence of DOM (i.e. compared to circles).

Fig. 2.

Antinociceptive effects of morphine alone and in combination with DOM in a warm water tail withdrawal procedure in rats (left) and in monkeys (right; replotted from Li et al, 2011). Abscissae: dose of morphine in milligrams per kilogram body weight; ordinate: average ($\pm$ SEM) tail flick latency from 50° C water expressed as a percentage of the maximal possible effect (20 seconds). Points above "V" represent the effects of saline (solid circles) or DOM alone. *= $P<0.05$ compared to "V" (center panel) or to the effects of morphine in the absence of DOM.

Fig. 3. Antinociceptive effects of 10.0 mg/kg morphine alone, in combination with 8-OH-DPAT, and in combination with both 8-OH-DPAT and WAY 100635 in a radiant heat tail flick procedure (left) and in a warm water tail withdrawal procedure (right). Abscissa: dose of 8-OH-DPAT in milligrams per kilogram body weight. $*$ = $P<0.05$ compared to the effect of 10.0 mg/kg morphine alone. $\#$ = $P<0.05$ compared to the effect of 10.0 mg/kg morphine in combination with the same dose of 8-OH-DPAT in the absence of WAY 100635. See Fig 1 for other details.

Fig. 4.

Discriminative stimulus effects of morphine alone and in combination with DOM (upper) or 8-OH-DPAT (lower) in rats (left) and in monkeys (right; replotted from Li et al, 2011). Abscissae: dose of morphine in milligrams per kilogram body weight. Ordinates: average percentage of responses made on the morphine-associated lever ($\pm$ SEM). "V" represents the effects of saline or single doses of DOM or 8-OH-DPAT alone; *= $P < 0.05$ compared to the effects of morphine alone.

Fig. 5.

Left panels: locomotor effects of morphine alone, in combination with DOM, and in combination with both DOM and MDL100907. Right panels: locomotor effects of morphine alone, in combination with 8-OH-DPAT, and in combination with both 8-OH-DPAT and WAY 100635. Abscissa: dose of morphine in milligrams per kilogram body weight. Ordinate: locomotor activity expressed as a percentage of locomotion observed after the administration of saline. *= $P < 0.05$ compared to the effects of morphine alone. #= $P < 0.05$ compared to the effects of morphine in combination with either DOM or 8-OH-DPAT.