Effects of 2,5-dimethoxy-4-methylamphetamine (DOM) and 2-piperazin-1-yl-quinoline (quipazine) on fentanyl versus food choice in rhesus monkeys

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ABSTRACT

There has been increasing interest in the potential therapeutic effects of drugs with agonist properties at serotonin 2A subtype (5-HT$_{2A}$) receptors (e.g., psychedelics), including treatment of substance use disorders. Studying interactions between 5-HT$_{2A}$ receptor agonists and other drugs is important for understanding potential therapeutic effects as well as adverse interactions. Direct-acting 5-HT$_{2A}$ receptor agonists such as 2,5-dimethoxy-4-methylamphetamine (DOM) and 2-piperazin-1-yl-quinoline (quipazine) enhance some (e.g. antinociceptive) effects of opioids; however, it is unclear whether they alter the abuse-related effects of opioids. This study examined whether DOM and quipazine alter the reinforcing effects of fentanyl in rhesus monkeys (n=6) responding under a food versus drug choice procedure. Responding on one lever delivered sucrose pellets and responding on the other lever delivered intravenous infusions. In one set of experiments, fentanyl (0.1-3.2 µg/kg/infusion) versus food choice sessions were preceded by noncontingent intravenous pretreatments with DOM (0.032-0.32 mg/kg), quipazine (0.32-1.0 mg/kg), naltrexone (0.032 mg/kg), or heroin (0.1 mg/kg). In another set of experiments, fentanyl was available during choice sessions in combination with DOM (0.32-100 µg/kg/infusion) or quipazine (3.2-320 µg/kg/infusion) in varying dose ratios. Naltrexone decreased and heroin increased fentanyl choice, demonstrating sensitivity of responding to pharmacological manipulation. However, whether given as a pretreatment or available in combination with fentanyl as a mixture, neither DOM nor quipazine significantly altered fentanyl choice. These results suggest that 5-HT$_{2A}$ receptor agonists do not enhance the reinforcing effects of opioids and, thus, will not likely enhance abuse potential.
SIGNIFICANCE STATEMENT

Serotonin 2A subtype receptor agonists enhance some (e.g., antinociceptive) effects of opioids, suggesting they could be combined with opioids in some therapeutic contexts such as treating pain. However, it is unclear whether they also enhance adverse effects of opioids, including abuse. Results of this study indicate that serotonin 2A subtype receptor agonists do not reliably enhance opioid self-administration and, thus, are unlikely to enhance the abuse potential of opioids.
INTRODUCTION

There has been substantial and growing interest in the potential therapeutic utility of drugs with agonist properties at serotonin 2A subtype (5-HT$_{2A}$) receptors (e.g., Belouin and Henningfield, 2018; Garcia-Romeu and Richards, 2018; Nichols et al., 2017; Nutt et al., 2020), including for treatment of substance use disorders (De Veen et al., 2017; DiVito and Leger, 2020; Dos Santos et al., 2018). Therefore, studying interactions between 5-HT$_{2A}$ receptor agonists and other drugs is important for evaluating their potential therapeutic utility. Interactions with opioids are of particular interest because of the steady rise in drug overdose deaths attributed in large part to opioids with rates that have reached record highs in recent years.

Although relatively few studies have been published, complex interactions between opioids and 5-HT$_{2A}$ receptor agonists have been reported. For example, in one study, 2,5-dimethoxy-4-methylamphetamine (DOM), 2-piperazin-1-yl-quinoline (quipazine), and 2,5-dimethoxy-4-n-propylthiophenethylamine (2C-T-7) enhanced the antinociceptive effects of morphine in rhesus monkeys, shifting the morphine dose-effect curve leftward (Li et al., 2011). The interaction was greater than additive and mediated, at least in part, by 5-HT$_{2A}$ receptors as leftward shifts in the morphine dose-effect curve were blocked by the 5-HT$_{2A}$ receptor antagonist MDL100907. In contrast to enhancement of antinociceptive effects, DOM, quipazine, and 2C-T-7 attenuated the discriminative stimulus effects of morphine in the same study, indicating that these drugs enhance some but not all effects of opioids. In contrast, when tested in rats, DOM failed to significantly alter the antinociceptive or discriminative stimulus effects of morphine (Li et al., 2013), suggesting a possible species difference. Moreover, in mice,
MDL100907 modestly enhanced the antinociceptive effects of oxycodone in male, but not female, mice (Sierra et al., 2022), and blocking 5-HT$_{2A}$ receptors with an antagonist suppressed behavioral sensitization and naloxone-precipitated withdrawal symptoms in morphine-treated mice (Li et al., 2021; Pang et al., 2016). Taken together, studies on interactions between opioids and 5-HT$_{2A}$ receptor agonists indicate a complex pattern of interactions warranting further elucidation.

Few studies have investigated interactions between opioids and 5-HT$_{2A}$ receptor agonists with regard to positive reinforcing effects that contribute to abuse. One study (Maguire et al., 2013) examined effects of DOM and quipazine on heroin self-administration in rhesus monkeys. Whether administered as a pretreatment or made available along with heroin in a mixture, neither drug reliably enhanced heroin self-administration, and, in some cases, decreased responding for heroin. A recent study (Martin et al., 2021) showed that 2,5-dimethoxy-4-iodoamphetamine (DOI), a 5-HT$_{2A}$ receptor agonist and congener of DOM, decreased economic demand for fentanyl in rats.

Although previous studies suggest 5-HT$_{2A}$ receptor agonists might decrease the reinforcing effects of opioids, these studies used single-lever self-administration procedures which can be subject to multiple interpretations including that decreased responding reflected generalized rate-depressant effects. Therefore, the current study extended this research by examining effects of DOM and quipazine on the reinforcing effects of fentanyl in rhesus monkeys responding under a food versus drug choice procedure which can disentangle effects of experimental manipulations on reinforcing effects of drugs from generalized rate-altering effects (e.g., Banks and Negus, 2017).
For example, under choice procedures, opioid receptor antagonists such as naltrexone and methocinnamox shifted allocation of behavior from responding for an opioid to responding for a non-drug alternative (food) in the absence of significant reductions in overall response output (e.g., Maguire et al. 2019), consistent with decreasing the relative reinforcing effects of drug.

In this study, monkeys could respond on one lever for food and on the other lever for an intravenous infusion. In one set of experiments, fentanyl versus food choice sessions were preceded by noncontingent intravenous pretreatments with DOM or quipazine. In another set of experiments, fentanyl was available during choice sessions in combination with DOM or quipazine in varying dose ratios. Acute, non-contingent pretreatments determined whether presence of drug per se modifies the reinforcing effects of fentanyl, whereas the drug mixture experiments evaluated the importance of response-contingent drug administration, since drugs used therapeutically would most likely be self-administered in combination. If DOM and quipazine enhance the positive reinforcing effects of fentanyl, then they would be expected to increased fentanyl choice over food (e.g., shift the fentanyl dose-effect curve leftward). On the other hand, if these drugs decrease the positive reinforcing effects of opioids, as suggested by previous studies, then they would be expected to decrease fentanyl choice and increase choice of food (e.g., shift the fentanyl dose-effect curve rightward or downward). Naltrexone and heroin served as positive control tests, demonstrating that responding under this choice procedure is sensitive to pharmacological manipulation.
METHODS AND MATERIALS

Subjects. Six adult rhesus monkeys (5 males and 1 female) were housed individually in stainless steel cages located in a colony room maintained under 14/10-hr light/dark cycle, with lights on at 0600 hr. The home cage also served as the experimental chamber as described in more detail below. Chow (High Protein Monkey Diet; Harlan Teklad, Madison, WI, USA), fresh fruit, peanuts, and other treats were provided daily, at least 30 min after experimental sessions, and water was continuously available. Experiments were conducted in accordance with guidelines set forth by the Guide for the Care and Use of Laboratory Animals (8th edition), and protocols were approved by the University of Texas Health Science Center at San Antonio Institutional Animal Care and Use Committee.

Surgery. Surgical procedures were similar to those described previously (e.g., Maguire and France, 2018; Maguire et al., 2019). Monkeys were sedated with ketamine (10 mg/kg, intramuscular; Henry Schein Animal Health, Dublin, OH, USA), intubated, and then maintained on 2 l/min oxygen and isoflurane anesthesia (Butler Animal Health Supply, Grand Prairie, TX). Under aseptic conditions, a 5-french polyurethane catheter (Access Technologies, Skokie, IL) was inserted into a vein and tunneled subcutaneously (s.c.) to an exit point in the mid-scapular region. Penicillin B&G and meloxicam were given postoperatively.

Apparatus. The exterior part of the catheter was passed through a flexible stainless steel tether and connected to a stainless steel fluid channel swivel secured to the back of the cage; monkeys wore a mesh jacket that protected the catheter and secured the tether (Lomir Biomedical, Quebec, Canada). Outside of the cage, the swivel
was connected by a catheter extension set to a syringe located in a syringe pump (PHM-108; Med Associates, Inc., Fairfax, VT, USA) that infused at a rate of 3.6 ml/min. Attached to one side of the cage was a custom-made stainless steel instrument panel (20 cm high by 28 cm wide) that contained 2 horizontally aligned response levers measuring 3.25 cm wide (ENV-610M; Med Associates) separated by 2 partitions that prevented pressing both levers with the same limb. Above each lever was a pair of horizontally aligned stimulus lights measuring 2.5 cm in diameter one of which could be illuminated green (ENV-221GN-LED; Med Associates); the other light was not used in this study. Raspberry flavored sucrose pellets (300 mg, 5TUT, Test Diet, Richmond, IN) were delivered via a pellet dispenser (ENV-203-300; Med Associates) through a 6 cm high by 5 cm wide aperture located directly above the instrument panel. Experimental events were programmed and data collected using a PC-compatible computer and Med-PC® IV software and associated interface equipment (Med Associates).

**Drugs.** Fentanyl hydrochloride, 3,6-diacetylmorphine (heroin) hydrochloride, (±) 2,5-dimethoxy-4-methylamphetamine (DOM) hydrochloride, and naltrexone hydrochloride were generously provided by the National Institute on Drug Abuse Drug Supply Program (Rockville, MD, USA); 2-piperazin-1-yl-quinoline (quipazine) dimaleate was purchased from Ascent Scientific (Bristol, UK). Doses were expressed as the salt; all drugs were dissolved in sterile saline and filtered (0.22 µm pore size) prior to administration. Fentanyl, DOM, and quipazine were infused i.v. during self-administration sessions in volumes ranging from 0.032 to 1 ml per 10 kg of body weight. Naltrexone, heroin, DOM, and quipazine were administered i.v. as pretreatments prior
to self-administration sessions in volumes of approximately 0.32 ml per 10 kg of body weight; pretreatments were followed by a 3-ml i.v. infusion of saline flush.

**Food versus drug choice procedure.** Before each session, a syringe containing the solution available for self-administration that day was attached to the catheter, and the pump was activated to fill the catheter. Sessions, conducted once daily 7 days per week, began at 1000 hr and comprised 4 blocks that were 40 min in duration with 20-min inter-block intervals. Each block began with 2 forced trials followed by up to 6 choice trials. During a forced trial, the green light above one lever was illuminated and 30 consecutive responses on the lever directly below turned off the light, delivered the reinforcer associated with that lever (one food pellet or an i.v. infusion), and initiated a 5-min timeout during which all lights were off and responding had no programmed consequence. At the end of the timeout, the green light over the other lever was illuminated and 30 consecutive responses on the lever located directly below turned off the light, delivered the reinforcer associated with that lever, and initiated a timeout. The order of forced trials varied randomly across blocks and sessions with the constraint that each block contained one right and one left forced trial.

Choice trials began once both forced trials were completed. Both green lights were illuminated and 30 consecutive responses on either lever produced the outcomes presented during the forced trials. Blocks ended after completion of 8 trials (2 forced and 6 choice), or 40 min, whichever occurred first; if all trials were completed, the post-reinforcer timeout was extended to include the remainder of the block. The unit dose of drug available for self-administration increased in half-log unit steps across blocks within the session by varying the infusion duration; otherwise, the stimuli presented
were identical across blocks. Responses on one lever reset the response requirement for the other lever; responding on one lever always delivered food while responding on the other lever always delivered an infusion. Lever designations were randomly determined across monkeys and remained constant for each monkey for the duration of the study. Catheter lines were flushed with 3 ml of heparinized saline (100 U/ml; Hospira Inc., Lake Forest, IL) after the session to promote catheter patency.

**Experimental design and timeline.** Several types of tests were conducted as outlined in Table 1; tests were conducted in the order in which they appear in the table. For pretreatment tests, an injection was administered i.v. 15 min prior to the start of the session (0945 hr), followed by a 3-ml saline flush, and before attaching a syringe of solution available for self-administration to the catheter. Test sessions occurred so long as 1) the total number of choice trials completed during each session for 3 consecutive sessions did not vary by more than 20% of the mean number of trials completed across those 3 sessions and 2) infusion choice for the entire session across those same 3 sessions did not vary by more than 20%. For drug substitution and mixture tests, saline alone, fentanyl alone, DOM alone, quipazine alone, or fentanyl in combination with either DOM or quipazine was available for self-administration for at least 3 and no more than 7 sessions and until responding was stable as indicated above.

Effects of DOM pretreatments were examined first across a range of doses (0.032, 0.1, and 0.32 mg/kg) shown to have behavioral activity in previous studies (Li et al., 2008; Li et al., 2009; Li et al., 2011; Maguire et al., 2013). Pretreatment with 0.1 mg/kg heroin and 0.032 mg/kg naltrexone were then tested; these doses of heroin and naltrexone have been shown to alter opioid self-administration in rhesus monkeys (e.g.,
Gerak and France, 2021; Maguire et al., 2020). Thereafter, potential reinforcing effects of DOM available alone were evaluated before studying effects of mixtures of fentanyl and DOM. After responding was stable with increasing unit doses of fentanyl, saline was substituted for fentanyl for all blocks of the session and until percent infusion choice in each block decreased to 20% or less for a single session. At that point, DOM was made available with unit dose (3.2, 10, 32 and 100 µg/kg/infusion) increasing across blocks of the session for at least 3 sessions and until responding was stable. These unit doses were chosen such that the total cumulative dose in the case of exclusive drug choice would exceed the largest dose given as a bolus injection during the pretreatment study, thus increasing the likelihood of testing behaviorally active doses. Thereafter, fentanyl alone was made available as before for at least 3 sessions and until responding was stable, at which point mixtures of fentanyl and DOM were tested by combining fentanyl and DOM in the solution available for self-administration. Ratios of fentanyl to DOM (1:3.2, 1:10, and 1:32) were tested in descending order with fentanyl alone being available between successive tests with each ratio.

Tests of quipazine pretreatments took the same approach as with DOM pretreatment with doses of quipazine (0.32 and 1.0 mg/kg) selected based on evidence of behavioral activity in previous studies (Li et al., 2008; Li et al., 2009; Li et al., 2011; Maguire et al., 2013). Thereafter, potential reinforcing effects of quipazine available alone, with unit dose (10, 32, 100, and 320 µg/kg/infusion) increasing across blocks of the session. Then, mixtures of fentanyl and quipazine were tested by combining fentanyl and quipazine in the solution available for self-administration. Ratios of fentanyl to quipazine (1:32 and 1:100) were tested in descending order with fentanyl alone being
available between successive tests with each ratio. For reasons unrelated to the study, one monkey was removed from the experiment prior to tests with quipazine; therefore, only 5 monkeys participated in these tests.

**Data analysis.** The primary dependent measures were percent infusion choice (the total number of choice trials completed on the infusion lever divided by the total number of the choice trials completed multiplied by 100), the total number of choice trials completed, and response rate (number of responses divided by the time a green light was illuminated). Data from sessions immediately preceding each test served as control sessions; for each type of test (pretreatment, mixture, etc) data from control sessions were averaged. Unless otherwise indicated, pretreatment and mixture data were analyzed using two-way, repeated measures ANOVA with unit dose of fentanyl as one factor and either pretreatment or mixture dose ratio as the other factor. Data for DOM or quipazine available for self-administration alone were also analyzed using a two-way, repeated measures ANOVA with drug (fentanyl, saline, and either DOM or quipazine) as one factor and dose or block (saline) as the other factor. A mixed-effects model was used to analyze percent infusion choice data in cases where some cells were blank due to failure to complete choice trials. A Greenhouse–Geisser adjustment was employed to correct for violations of sphericity, and post-hoc comparisons made using the Bonferroni’s multiple comparisons test. Analyses were conducted using GraphPad Prism version 9.3.1 (GraphPad Prism Software, LLC, San Diego, CA).

**RESULTS**

Choice of infusions increased with increasing unit doses of fentanyl (Figure 1A, triangles). Monkeys chose fentanyl on fewer than 10% of trials with unit doses of 0.1
and 0.32 µg/kg/infusion and on at least 80% of trials with unit doses of 1.0 and 3.2 µg/kg/infusion. On average, at least 5 choice trials were completed each block (Figure 1B) and response rate ranged from 1.4 to 3.1 responses per second across blocks (Figure 1C).

Pretreatment with 0.032 mg/kg naltrexone shifted the fentanyl dose-effect curve rightward, significantly decreasing fentanyl choice at a unit dose of 1.0 µg/kg/infusion from greater than 90% to less than 15% (squares, Figure 1A) and increasing response rate with 3.2 µg/kg/infusion of fentanyl (Figure 1C) without significantly altering choice trials completed (Figure 1B). For percent infusion choice, there was a significant main effect of fentanyl unit dose (F(1.53, 7.67)=357.2, p<.0001), a significant main effect of naltrexone pretreatment (F(1.0, 5.0)=23.9, p=0.005), and a significant fentanyl by naltrexone interaction (F(1.38, 6.91)=22.18, p=0.002). For response rate, there was a significant fentanyl by naltrexone interaction (F(1.87, 9.37) = 4.1, p<.046) but no main effect of fentanyl dose or naltrexone pretreatment. There was no significant effect on choice trials completed.

Pretreatment with 0.1 mg/kg heroin shifted the fentanyl dose-effect upward, significantly increasing percent fentanyl choice at a unit dose of 0.1 µg/kg/infusion from less than 5% to greater than 60%, on average (filled triangles, Figure 1D), without significantly altering choice trials completed (Figure 1E) or response rate (Figure 1F). For percent infusion choice, a mixed-effects model indicated a significant main effect of fentanyl unit dose (F(1.66, 8.31)=18.35, p=0.001), a significant main effect of heroin pretreatment (F(1.0, 5.0)=7.37, p=0.04), and a significant fentanyl by heroin interaction (F(1.51, 6.52)=7.39, p=0.03). There was a significant effect of fentanyl dose on
response rate ($F(2.39, 11.98)=7.19, p=0.007$) but no main effect of heroin or fentanyl by heroin interaction, and there were no significant effects on choice trials completed.

Pretreatment with DOM did not significantly alter percent fentanyl choice (Figure 2A), although it dose-dependently decreased the number of choice trials completed (Figure 2B) and response rate (Figure 2C). For percent infusion choice, a mixed-effects model indicated a significant main effect of fentanyl unit dose ($F(1.28,6.40)=62.52, p=0.0001$) but no main effect of DOM pretreatment or interaction. For choice trials completed, there was a significant main effect of fentanyl unit dose ($F(1.54,7.69)=4.87, p=0.049$), a significant main effect of DOM pretreatment ($F(1.46,7.29)=7.86, p=0.02$), and a significant interaction between fentanyl unit dose and DOM pretreatment ($F(2.22,11.10)=3.93, p=0.048$). For response rate, there was a significant main effect of fentanyl unit dose ($F(1.76,8.79)=4.59, p=0.046$), a significant main effect of DOM pretreatment ($F(1.37,6.86)=11.34, p=0.009$), and a significant interaction between fentanyl unit dose and DOM pretreatment ($F(3.32,16.60)=5.0, p=0.01$).

When available alone, fentanyl but neither DOM nor saline significantly increased percent infusion choice (Figure 2D) and decreased response rate (Figure 2F). For percent choice, there was a significant main effect of dose ($F(1.71,8.56)=38.27, p<0.0001$), and drug ($F(1.22,6.12)=98.73, p<0.0001$), as well as a drug by dose interaction ($F(1.50,7.52)=37.62, p=0.0002$) with only 1.0 and 3.2 µg/kg/infusion of fentanyl differing from saline. There was also a significant dose by drug interaction for response rate ($F(2.86,14.31)=4.16, p=0.03$), with 3.2 µg/kg/infusion of fentanyl differing from saline. Combining DOM with fentanyl as a mixture available for self-administration did not significantly alter percent fentanyl choice (Figure 2G), although it significantly
decreased the number of choice trials completed (Figure 2H). For percent infusion choice, there was a significant main effect of fentanyl unit dose (F(1.65, 8.24)=121.5, p<0.0001) but no main effect of the DOM mixture or interaction. For trials completed there was a significant main effect of DOM mixture (F(1.95, 9.73)=7.84, p=0.01) and a significant fentanyl dose by DOM mixture interaction (F(2.35,11.77)=3.77, p=0.049) but no main effect of fentanyl dose. For response rate, there was a significant main effect of fentanyl dose (F(2.38,11.92)=9.92, p=0.002) but no main effect of DOM mixture or interaction.

Pretreatment with quipazine did not significantly alter percent fentanyl choice (Figure 3A), the number of choice trials completed (Figure 3B), or response rate (Figure 3C). For percent infusion choice, a mixed-effects model indicated a significant main effect of fentanyl dose (F(1.96,7.84)=59.11, p<0.0001) but no main effect of quipazine pretreatment or interaction. Although pretreatment with 1.0 mg/kg quipazine tended to decrease the number of choice trials completed and response rate, these decreases did not reach statistical significance.

When available alone, fentanyl but neither quipazine nor saline significantly increased percent infusion choice (Figure 3D). For percent choice, there was a significant main effect of dose (F(1.20,4.80)=96.89, p=0.0002), and drug (F(1.59,6.37)=149.4, p<0.0001), as well as a drug by dose interaction (F(2.14,8.58)=48.34, p<0.0001) with only 1.0 and 3.2 µg/kg/infusion of fentanyl differing from saline. There were no significant effects for trials completed or response rate. Combining quipazine with fentanyl as a mixture available for self-administration did not significantly alter percent fentanyl choice (Figure 3G), choice trials completed (Figure
3H), or response rate (3I). For percent infusion choice, there was a significant main effect of fentanyl unit dose (F(2.01,8.02)=203.8, p<0.0001) but no main effect of the quipazine mixture or interaction. There were no significant effects for trials completed, and for response rate, there was a significant main effect of fentanyl dose (F(1.48,5.91)=8.74, p=0.02) but no main effect of quipazine mixture or interaction.

DISCUSSION

This study examined effects of DOM and quipazine, two drugs with 5-HT\textsubscript{2A} receptor agonist properties, on the positive reinforcing effects of fentanyl in rhesus monkeys responding under a food versus drug choice procedure. Whether given non-contingently as a pretreatment or made available along with fentanyl as a mixture, neither DOM nor quipazine significantly altered choice of fentanyl, suggesting that drugs with agonist properties at 5-HT\textsubscript{2A} receptors likely do not reliably alter the positive reinforcing effects of opioids. On the other hand, pretreatment with naltrexone or heroin significantly attenuated and enhanced, respectively, fentanyl choice demonstrating sensitivity of responding under this procedure to pharmacological manipulation.

Fentanyl dose dependently increased choice of infusions over food with unit doses of 1.0 and 3.2 µg/kg/infusion of fentanyl maintaining over 80% drug choice. The potency of fentanyl to maintain drug choice was comparable to previous studies in rhesus monkeys using a food versus drug choice procedure (Townsend et al., 2021) as well as studies employing single-lever self-administration procedures (e.g., Broadbear et al., 2004; Maguire et al., 2020; Negus et al., 2008). When saline was substituted for fentanyl, choice of infusions across all blocks of the session quickly decreased to less
than 20% demonstrating sensitivity of the procedure to the reinforcing effects of drug infusions. When available alone, neither DOM nor quipazine increased choice of drug over food. The failure of both drugs to increase drug choice is consistent with numerous reports that 5-HT$_{2A}$ agonists do not reliably exhibit reinforcing or rewarding effects in nonhuman subjects, as demonstrated by failure to maintain responding under self-administration procedures (e.g., Deneau et al., 1969; Fantegrossi et al., 2004; Maguire et al., 2013; Yanagita, 1986; see Goodwin, 2016 and Siegel and Jarvik, 1980 for rare exceptions) and failure to decrease threshold under intra-cranial self-stimulation procedures (e.g., Sakloth et al., 2019). Moreover, the lack of robust rewarding effects under several preclinical procedures is consistent with assessments that 5-HT$_{2A}$ agonists likely have very low or no abuse potential relative to other drugs in humans (e.g., Johnson et al., 2018; Heal et al., 2018).

DOM and quipazine failed to reliably alter choice of fentanyl over food, although there was a tendency for DOM pretreatment to shift the fentanyl dose-effect rightward. Indeed, in 5 out of 6 monkeys, at least one dose of DOM (but no dose of quipazine) decreased fentanyl choice the third block of the session when 1.0 µg/kg/infusion fentanyl was available. However, effects on choice did not reach statistical significance and occurred at doses of DOM that also substantially decreased response rate, suggesting that other effects might have played a role. On rare occasions DOM (one dose in one monkey) and quipazine (one dose in another monkey) increased choice of fentanyl in the first block of the session when 0.1 µg/kg/infusion fentanyl was available; however, these changes also occurred along with substantial decreases in response rate. Thus, there was no reliable evidence that DOM or quipazine altered the reinforcing
effects of fentanyl independent of marked changes in overall response output. The 5-HT\textsubscript{2A} receptor agonist LSD has been reported to function as an aversive stimulus under some conditions (e.g., Hoffmeister, 1975). Therefore, it might be expected that response-contingent delivery of other 5-HT\textsubscript{2A} receptor agonists such as DOM or quipazine would function as punishers; that is, response-contingent presentation of drug would decrease responding for another reinforcer. In this case, DOM or quipazine might have decreased choice of fentanyl as is the case with other drug punishers such as histamine (e.g., Negus, 2005). However, failure of response-contingent delivery of DOM or quipazine to decrease fentanyl choice suggests that neither drug exhibited robust punishing effects either. Whether aversive effects of response-continent presentation of these drugs emerge under other procedures has yet to be determined.

DOM and quipazine pretreatment tests were conducted up to doses that decreased rate of responding. Although decreases following quipazine pretreatment were not statistically significant, there was a clear decrease in 3 of the 5 subjects. Therefore, failure of both drugs to significantly alter choice of fentanyl is likely not due to insufficient doses. Moreover, the doses administered were in the range of those studied previously in rhesus monkeys and shown to be behaviorally active (Li et al., 2008; Li et al., 2009; Li et al., 2011; Maguire et al., 2013). In contrast to DOM and quipazine, pretreatment with naltrexone attenuated and heroin enhanced fentanyl choice. Naltrexone attenuated fentanyl choice, shifting the dose-effect curve rightward, without substantially altering the number of trials completed or response rate, consistent with its antagonist properties at \textit{mu} opioid receptors. The pretreatment dose of naltrexone has been shown to reliably decrease fentanyl-maintained responding previously (Maguire et
al., 2019; Maguire et al., 2020; Maguire and France 2022). Conversely, pretreatment with heroin increased choice of fentanyl, shifting the dose-effect curve up, consistent with a recent study demonstrating that mu opioid receptor agonists such as heroin and buprenorphine can increase choice of infusions over food in monkeys responding under similar conditions (Gerak and France, 2021). These tests demonstrated sensitivity of responding under this procedure to pharmacological manipulations.

DOM and quipazine have partial agonist properties at 5-HT$_{2A}$ receptors (e.g., Berg et al., 1998; Conn and Sanders-Bush 1987; Sanders-Bush et al., 1988) and share many behavioral effects with other 5-HT$_{2A}$ receptor agonists, including discriminative stimulus effects (e.g., Colpaert et al., 1981; Friedman et al., 1984; Glennon et al., 1983; Li et al., 2008; White et al., 1979) and ability to elicit head twitch responses in rodents (e.g., de la Fuente Revenga et al., 2021; Halberstadt et al., 2020; Vetulani et al., 1980). However, they have relatively high affinity for other targets including other serotonergic targets such as 5-HT$_{2C}$ subtype receptors. The pharmacological mechanism(s) contributing to the rate decreasing effects observed in this study were not confirmed in the current study. Nevertheless, both drugs exhibit prominent 5-HT$_{2A}$ receptor agonist activity at the doses tested in rhesus monkeys (Li et al., 2008; 2009) and failed to significantly alter the reinforcing effects of fentanyl despite such activity. Whether actions of DOM or quipazine at other pharmacological targets interfered with their potential to modify the reinforcing effects of fentanyl remains unclear.

The failure of DOM or quipazine to alter the positive reinforcing effects of fentanyl in the current study contrasts published data indicating that both drugs enhanced the antinociceptive effects and attenuated the discriminative stimulus effects of morphine in
rhesus monkeys in a 5-HT$_{2A}$ receptor-mediated manner (Li et al., 2011). Differential effects across procedures suggests that interactions are likely not the result of pharmacokinetic changes which might be expected to modify the effects of opioids similarly across procedures. DOM and quipazine tended to decrease heroin self-administration in rhesus monkeys (Maguire et al., 2013) while DOI decreased demand for fentanyl in rats (Martin et al., 2021) which could be interpreted as attenuation of the positive reinforcing effects. The current results are at odds with these previous reports. One possibility is that the procedures employed (food/drug choice in the current study versus single-response procedures used previously) are differentially sensitive to various factors that can alter drug self-administration. Data from the current experiment which examined changes in response allocation rather than overall response output suggests that effects previously reported might have been due, at least in part, to non-selective response rate-depressant effects. Additional studies directly comparing effects of pharmacological manipulations on drug self-administration maintained under different procedural variations are required to fully characterize which factor(s) might play a role in such discrepancies.

Data from the current study, taken together with previous reports, suggest that 5-HT$_{2A}$ receptor agonists do not enhance the discriminative stimulus or positive reinforcing effects of opioids, effects that are thought to be reflective of their abuse potential. Thus, combining 5-HT$_{2A}$ receptor agonists with opioids for therapeutic purposes (e.g., treating pain) likely will not enhance abuse. However, it is important to determine whether 5-HT$_{2A}$ receptor agonists enhance other adverse effects of opioids, such as respiratory depression and the development of tolerance and physical
dependence, in order to fully characterize interactions between these drugs and evaluate the potential therapeutic utility of drug combinations.
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AUTHORSHIP CONTRIBUTIONS

Participated in research design: Maguire, D.
Conducted experiments: Maguire, D.
Performed data analyses: Maguire, D.
Wrote or contributed to the writing of the manuscript: Maguire, D.
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FOOTNOTES

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FIGURE LEGENDS

**Figure 1.** Effects of pretreatment with either naltrexone or heroin in rhesus monkeys responding under a food versus drug choice procedure. Percent infusion choice (top row), total choice trials completed (middle row), and response rate (bottom row) are plotted as a function of the unit dose of fentanyl. The unit dose of fentanyl increased across blocks within each session. Control data are indicated by open triangles, whereas data following pretreatment with 0.032 mg/kg naltrexone or 0.1 mg/kg heroin, given i.v. 15 min before the session, are indicated by filled squares (left column; panels A, B, and C) and filled triangles (right column; panels D, E, and F), respectively. Symbols show the mean (n=6) and error bars indicate the standard error of the mean. Asterisks indicate data following pretreatment that differ from control according to a Bonferroni’s post-hoc test.

**Figure 2.** Effects of DOM (given as a pretreatment, available alone, or combined with fentanyl) in monkeys responding under a food versus drug choice procedure. Percent infusion choice (top row), total choice trials completed (middle row), and response rate (bottom row) are plotted as a function of unit dose. Panels in the left column show effects of DOM pretreatment; control data are shown by open triangles, whereas filled symbols show data from different doses of DOM administered i.v. 15 min prior to the session. Panels in the center column show data from responding when fentanyl, saline, or DOM were available alone for self-administration. Panels in the right column show data when DOM and fentanyl were available for self-administration as a mixture. For all panels, symbols show the mean (n=6) and error bars indicate the standard error of the mean.
mean. Asterisks indicate data following pretreatment that differ from control (left and right columns) or saline (middle column) according to a Bonferroni’s post-hoc test.

**Figure 3.** Effects of quipazine (given as a pretreatment, available alone, or combined with fentanyl) in monkeys responding under a food versus drug choice procedure. Percent infusion choice (top row), total choice trials completed (middle row), and response rate (bottom row) are plotted as a function of the unit dose. Panels in the left column show effects of quipazine pretreatment. Control data are shown by open triangles, whereas filled symbols show data from different doses of quipazine administered i.v. 15 min prior to the session. Panels in the center column show data from responding when fentanyl, saline, or quipazine were available alone for self-administration. Panels in the right column show data from responding when quipazine and fentanyl were available for self-administration as a mixture. For all panels, symbols show the mean (n=5) and error bars indicate the standard error of the mean. Asterisks indicate data following pretreatment that differ from control (left and right columns) or saline (middle column) according to a Bonferroni’s post-hoc test.
**TABLES**

**Table 1.** Experimental conditions and test doses. Tests were conducted in the order in which they appear in the table.

<table>
<thead>
<tr>
<th>Experimental condition</th>
<th>Doses(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DOM pretreatment(^b)</td>
<td>0.032, 0.1, and 0.32 mg/kg</td>
</tr>
<tr>
<td>Heroin pretreatment(^b)</td>
<td>0.1 mg/kg</td>
</tr>
<tr>
<td>Naltrexone pretreatment(^b)</td>
<td>0.032 mg/kg</td>
</tr>
<tr>
<td>DOM alone substitution(^c)</td>
<td>3.2, 10, 32, and 100 µg/kg/infusion</td>
</tr>
<tr>
<td>DOM mixture (fentanyl:DOM ratio)(^b,c)</td>
<td></td>
</tr>
<tr>
<td>1:3.2</td>
<td>0.32, 1.0, 3.2, and 10 µg/kg/infusion</td>
</tr>
<tr>
<td>1:10</td>
<td>1.0, 3.2, 10, and 32 µg/kg/infusion</td>
</tr>
<tr>
<td>1:32</td>
<td>3.2, 10, 32, and 100 µg/kg/infusion</td>
</tr>
<tr>
<td>Quipazine pretreatment(^b)</td>
<td>0.32 and 1.0 mg/kg</td>
</tr>
<tr>
<td>Quipazine alone substitution(^c)</td>
<td>10, 32, 100, and 320 µg/kg/infusion</td>
</tr>
<tr>
<td>Quipazine mixture (fentanyl:quipazine ratio)(^b,c)</td>
<td></td>
</tr>
<tr>
<td>1:32</td>
<td>3.2, 10, 32, and 100 µg/kg/infusion</td>
</tr>
<tr>
<td>1:100</td>
<td>10, 32, 100, and 320 µg/kg/infusion</td>
</tr>
</tbody>
</table>

\(^a\)Pretreatment doses are expressed in mg/kg whereas self-administered unit doses are expressed in µg/kg/infusion

\(^b\)Fentanyl unit doses of 0.1, 0.32, 1.0, and 3.2 µg/kg/infusion were available in ascending order across blocks of the session

\(^c\)The unit doses of DOM or quipazine indicated were available in ascending order across blocks of the session
Figure 2

Pretreatment

A

% Infusion choice

DOM (mg/kg)

\(\bigtriangleup\) 0

\(\square\) 0.032

\(\bigtriangleup\) 0.1

\(\downarrow\) 0.32

B

Total choices

C

Responses per second

0.1 0.32 1.0 3.2

Fentanyl (μg/kg/infusion)

Drugs alone

D

% Infusion choice

Fentanyl

\(\bigtriangleup\)

Saline

\(\bigcirc\)

DOM

\(\square\)

* p < 0.05

E

Total choices

F

Responses per second

0.1 0.32 1.0 3.2

Fentanyl alone

DOM alone

Saline

G

% Infusion choice

Fentanyl: DOM

BL

1:3.2

1:10

1:32

H

Total choices

I

Responses per second

0.1 0.32 1.0 3.2

Fentanyl

0.32 1.0 3.2 10 32 100

DOM (1:3)

DOM (1:10)

DOM (1:30)

Dose (μg/kg/infusion)