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Pharmacology, Biochemistry and Behavior 75 (2003) 363–371
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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# The modulatory actions of dopamine D2/3 agonists and antagonists on the locomotor-activating effects of morphine and caffeine in mice

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Received 17 January 2003; received in revised form 17 April 2003; accepted 21 April 2003

**Abstract**

Morphine produces robust increases in locomotor activity in mice. Recent data indicate that dopamine (DA) D2/3 agonists attenuate the discriminative stimulus and antinociceptive effects of mu opioid agonists such as morphine. The present study was designed to determine the extent to which D2/3 receptor activation and blockade can modulate morphine-induced locomotion using a novel cumulative dosing procedure in Swiss–Webster mice. The results indicate that morphine-induced locomotion is nonsignificantly attenuated by the D2/3 agonists quinelorane and quinpirole, whereas the D2/3 antagonists eticlopride and nafadotride, as well as the partial D2/3 agonist BP897, significantly reduced morphine-induced locomotion. To determine the specificity of this modulation, these agonists and antagonists were examined in combination with caffeine, a drug that also indirectly alters DAergic activity. Unlike the effects on morphine, caffeine-induced locomotion was unaltered by eticlopride, nafadotride and BP897, but was attenuated by quinelorane and quinpirole. These results indicate that modulation of D2/3 receptors can, in turn, alter the locomotor-activating effects of morphine.


*Keywords:* Mice; Locomotor activity; Dopamine agonist; Dopamine antagonist; Mu opioid; BP897

## 1. Introduction

Neurochemical evidence indicates that the acute administration of mu opioid agonists results in stimulating dopamine (DA) release and turnover in the striatum and nucleus accumbens of rodents (Di Chiara and Imperato, 1988; Wise et al., 1995; Wood et al., 1983). This neurochemical interaction between the mu opioid system and the DA system has led many investigators to examine the role of DA and its receptors in modulating the behavioral effects of mu opioids. For instance, the ability of DA antagonists to modulate the reinforcing properties (David et al., 2002; Gerrits et al., 1994; Hemby et al., 1996; Shippenberg et al., 1993; Shippenberg and Herz, 1988; Winger et al., 1992) and discriminative stimulus effects (Colpaert et al., 1977; Cook and Picker, 1998; Corrigall and Coen, 1990; McCarten and Lal, 1979; Ukai et al., 1991) of the mu opioids heroin and morphine have been explored. The results from these studies vary in that under some conditions the reinforcing and discriminative stimulus effects are attenuated (e.g., Corrigall and Coen, 1990; Hemby et al., 1996; Shippenberg and Herz, 1988) or not affected (e.g., Shippenberg et al., 1993; Shippenberg and Herz, 1988; Ukai et al., 1991; Winger et al., 1992) by DA receptor antagonism. Besides having discriminative stimulus and reinforcing effects, mu opioid receptor agonists like morphine also have locomotor-activating effects (Brase et al., 1977; Kuschinsky and Hornykiewicz, 1974). Morphine-induced locomotion is attenuated by the coadministration of the DA D1 antagonist SCH23390 and the DA D2/3 antagonist sulpiride (Zarrindast and Zarghi, 1992), although interpretation of these results is equivocal because the effects of these antagonists administered by themselves on locomotor activity were not reported. Similarly, U-99194A, a D2/3 antagonist (Manzanedo et al., 1999), SCH23390, a D1 antagonist, and raclopride, a D2/3 antagonist, also attenuate the locomotor-activating effects of morphine; however, the attenuation by these latter agents occurs at doses that significantly

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doi:10.1016/S0091-3057(03)00090-X

suppress locomotion by themselves (Rodriguez-Arias et al., 2000). These studies indicate that DA antagonists, under some conditions, can attenuate morphine-induced locomotion. However, because DA antagonists suppress locomotor activity when administered by themselves (e.g., Chausmer and Katz, 2001; Fujiwara, 1992), drawing inferences about the modulatory role of DA from the effects of DA antagonists on morphine-induced locomotion can be complicated.

A previous study demonstrated that the D2/3 agonist 7-OH-DPAT attenuates morphine-induced locomotion in mice (Suzuki et al., 1995). More recently, we have shown that D2/3 receptor agonists attenuate the discriminative stimulus (Cook and Beardsley, 2001; Cook and Picker, 1998) and antinociceptive (Cook et al., 1999) effects of morphine. Taken together, these data suggest that D2/3 receptor activation can alter the behavioral effects of the mu opioid morphine. The present study was designed to evaluate the modulatory actions of D2/3 receptors on morphine-induced locomotion. The ability of D2/3 agonists (quinelorane and quinpirole) (Levant, 1997) and D2/3 antagonists (eticlopride and nafadotride) (Levant, 1997) as well as the partial D2/3 agonist BP897 (Pilla et al., 1999; Wicke and Garcia-Ladona, 2001; Wood et al., 2000) to affect morphine-induced locomotion was examined in male Swiss–Webster mice.

Opioids are not the only drug class known to indirectly alter DA brain levels. For example, caffeine, which is a methylxanthine, indirectly alters DAergic activity through antagonism of adenosine receptors (Acquas et al., 2002; Daly et al., 1981; Snyder et al., 1981). As with morphine, these alterations in DA are thought to mediate several of the behavioral effects of caffeine (e.g., locomotor-activating, discriminative stimulus and reinforcing effects) (Garrett and Griffiths, 1997). Although DA is thought to be intimately involved in the behavioral effects of both caffeine and morphine, it is not known whether DA agonists and antagonists modulate morphine- and caffeine-induced locomotion in similar manners. Therefore, caffeine was tested alone and in combination with selected doses of the D2/3 agonists and antagonists to determine the specificity of the effects obtained with morphine.

In most locomotor activity studies a mouse is exposed to only one dose of a drug, with activity levels being measured over a predetermined amount of time. This type of design requires the use of many subjects because separate groups of mice must be used for each dose to obtain a complete dose–effect curve. Moreover, when drug combination tests are conducted using this procedure the ability of several doses of a test drug (e.g., DA antagonist) to attenuate the effects of multiple doses of a standard drug (e.g., morphine) must be determined in separate groups of mice. Therefore, a cumulative dosing procedure was employed in this study such that the effect of a single dose of a test compound could be determined in combination with multiple doses of a standard like morphine in the same animal.

## 2. Method

### 2.1. Subjects

Adult male Swiss–Webster mice (Harlan Sprague–Dawley, Indianapolis, IN) weighing 25–35 g were used. Mice were housed five per cage, had continuous access to food and water, and were allowed to acclimate to the vivarium environment 1 week before the start of any testing. The vivarium was temperature controlled (22–24 °C) on a 12-h light–dark cycle. All testing occurred during the light component. Animals used in this study were cared for in accordance with the guidelines of the Institutional Animal Care and Use Committee of Virginia Commonwealth University, and the "Guide for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources, National Academy Press, 1996).

### 2.2. Cumulative locomotor activity procedure

Four commercially obtained, automated, activity-monitoring devices each enclosed in sound- and light-attenuating chambers were used (AccuScan Instruments, Columbus, OH). The interior of each chamber was divided into two separate 20 × 20 × 30-cm arenas permitting the independent and simultaneous measurement of two mice. Sixteen photobeam sensors were spaced 2.5 cm apart along the walls of the chamber. On each test day, eight mice were randomly selected from the available stock in the vivarium and brought to the laboratory where they were allowed to acclimate for approximately 30 min.

Each test session consisted of up to eight 15-min components. Each mouse was tested only on a single occasion. During the first component each mouse, regardless of group assignment, was placed into an activity chamber for a 15-min period during which activity was recorded but not used in the data analyses. Between components the mice were removed from their activity chambers, given one or two injections (see below), and immediately returned to their test chambers. The injection before the second component (i.e., between the end of the first and beginning of the second component) for all mice was of water vehicle. The activity level during the second component was used as the "baseline control" for each mouse. Separate groups of mice received different injections following each subsequent component. The water-only group continued to receive an injection of water immediately before each subsequent component. Some groups of mice received a single, high-dose injection of a D2/D3 agonist or antagonist preceding the third component and water injections preceding Components 4–7. Other groups of mice were given injections of either morphine or caffeine before Components 3–8 resulting in a cumulative dosing of each drug. Morphine-treated mice were given acute doses of 10, 20, 70, 30, 70 and 100 mg/kg morphine to result in cumulative doses of 10, 30, 100, 130, 200 and 300 mg/kg preceding the third through

eighth components, respectively. Caffeine-treated mice were tested for six 15-min components and were given acute doses of 3, 7, 20 and 70 mg/kg caffeine to result in cumulative doses of 3, 10, 30 and 100 mg/kg. Other groups of mice were treated with morphine or caffeine in a similar manner but, in addition, were given an injection of a D2/D3 agonist or antagonist along with the morphine (10 mg/kg) or caffeine (3.0 mg/kg) injection immediately before the third component.

### 2.3. Data analyses

Total distance traveled (centimeters) was assessed for each mouse during each 15-min component. Differences between water baseline control (second component) for the morphine-alone group and the morphine plus a D2/3 agonist or antagonist group were determined by an ANOVA. The effect of drug treatment (D2/3 agonist or antagonist) on morphine- or caffeine-induced locomotion was determined by a repeated measures ANOVA in which cumulative morphine or caffeine dose served as the within-subjects repeated measure and drug treatment (D2/3 agonist or antagonist) served as the between-subjects measure. ANOVA indicated that the water control baseline levels of activity differed amongst the BP897/morphine treatment groups. Therefore, an ANCOVA was conducted for BP897 in combination with morphine to control for these differences in baseline levels of activity. A Bonferroni post hoc adjustment was used for pairwise comparisons. The water baseline control (second component) for the water-alone group was compared with the water baseline control for the D2/3 agonist or antagonist group using an independent-samples $t$ statistic. Similarly, the water baseline control (second component) for the caffeine-alone group was compared with the water baseline control for the caffeine plus a D2/3 agonist or antagonist group using an independent-samples $t$ statistic. The alpha level for all comparisons was set at $P \leq .05$.

### 2.4. Drugs

Quinelorane dihydrochloride, (−)-quinpirole hydrochloride, (−)-eticlopride hydrochloride and caffeine were purchased from Sigma-Aldrich (St. Louis, MO). Nafadotride and BP897 were generously supplied by Pierre Sokoloff (Unite de Neurobiologie et Pharmacologie Moleculaire, INSERM, Paris, France). Morphine sulfate was provided by the National Institute on Drug Abuse (Research Triangle Institute, Cary, NC). All drugs were dissolved in sterile water except for BP897. BP897 was first dissolved in 20% wt/vol hydroxypropyl-β-cyclodextrin (ENCAPSIN HPB, Cerestar USA, Hammond, IN) in sterile water, at a maximal concentration of 5 mg/ml, to form a stock solution. Appropriate amounts of stock solution were added to sterile water to form test doses. All drugs were administered subcutaneously, except for quinelorane, quinpirole and caffeine, which were administered intraperitoneally. All drugs were administered in an injection volume of 5.0 or 10 ml/kg. Injections during tests in which a D2/3 agonist or antagonist was administered in combination with morphine (or caffeine), were injected separately and consecutively with the mice subsequently returned to the activity chambers.

## 3. Results

### 3.1. Effects of morphine alone and in combination with quinelorane and quinpirole

Fig. 1 shows the effects of morphine alone on total distance traveled as well as in combination with quinelorane (left panel) and quinpirole (right panel). There was a main effect of morphine dose for total distance traveled [$F(6,42) = 38.72$, $P \leq .05$]. A Bonferroni post hoc test indicated that relative to water control levels, a dose of 10 mg/kg morphine significantly reduced activity, whereas doses of

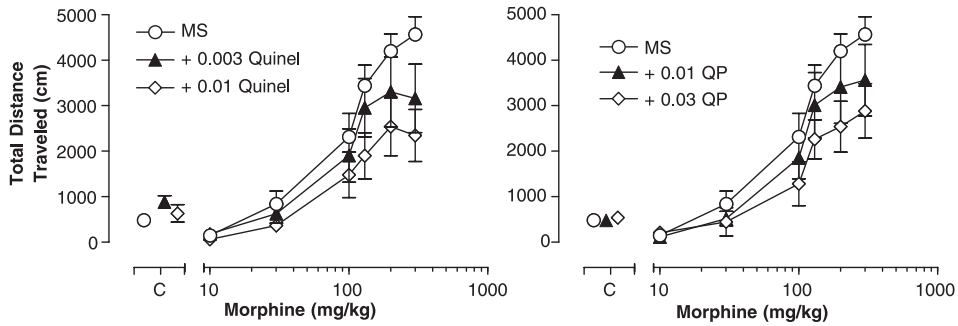


Fig. 1. Effects of morphine alone (MS, $n = 8$) and in combination with quinelorane (Quinel, $n = 8$) (left panel) and quinpirole (QP, $n = 8$) (right panel) on total distance traveled (cm). Data points above "C" represent the total distance traveled following water administration during the second 15-min component. Following the second component, mice received either (1) a morphine injection (10 mg/kg) or (2) an injection of morphine (10 mg/kg) in combination with a dose of an agonist at the beginning of the third component. For the remaining components all mice received morphine injections. The morphine curve represented in each panel is the same. Each data point represents the mean total distance traveled during each 15-min component. Brackets indicate S.E.M. Where brackets are not visible, the S.E.M. fell within the data point.

130, 200 and 300 mg/kg produced significant increases. An ANOVA indicated that water control values obtained during the second component were not different between the morphine-alone group and morphine plus quinelorane group [$F(2,21) = 2.09$, $P > .05$]. An ANOVA indicated that quinelorane treatment nonsignificantly decreased the effects of morphine [$F(2,21) = 2.29$, $P > .05$]. The ANOVA indicated that water control values obtained during the second component were not different between the morphine-alone group and morphine plus quinpirole group [$F(2,21) = 0.00$, $P > .05$]. Quinpirole nonsignificantly decreased the effects of morphine similar to that of quinelorane, [$F(2,21) = 1.28$, $P > .05$] (right panel).

### 3.2. Effects of morphine alone and in combination with eticlopride, nafadotride and BP897

Fig. 2 shows the effects of morphine alone on total distance traveled as well as in combination with eticlopride (left panel), nafadotride (middle panel) and BP897 (right panel). An ANOVA indicated that water control values obtained during the second component were not different between the morphine-alone group and the morphine plus eticlopride group [$F(2,21) = 1.01$, $P > .05$]. An ANOVA indicated there was a main effect of eticlopride treatment [$F(2,21) = 26.43$, $P \leq .05$] and a morphine dose by eticlopride treatment interaction [$F(10,105) = 18.63$, $P \leq .05$] such that a dose of 0.01 mg/kg eticlopride attenuated the effects of 300 mg/kg morphine and a dose of 0.03 mg/kg eticlopride attenuated the effects of 100–300 mg/kg morphine (left panel). An ANOVA indicated that water control values obtained during the second component were not different between the morphine-alone group and the morphine plus nafadotride group [$F(2,21) = 1.44$, $P > .05$]. An ANOVA indicated there was a main effect of nafadotride treatment [$F(2,21) = 5.36$, $P \leq .05$] and a morphine dose by nafadotride treatment interaction [$F(10,105) = 2.89$, $P \leq .05$] such that a dose of 1.0 mg/kg nafadotride attenuated the effects of 130 and 200 mg/kg morphine (middle panel). An ANOVA indicated that water control values obtained during the second component were different between the morphine-alone group and the morphine plus BP897 group [$F(2,21) = 4.41$, $P \leq .05$] such that the water control value for the group receiving morphine plus 0.3 mg/kg BP897 was significantly greater than the control value for the morphine-alone group, but not significantly different than the water control value for the morphine plus 1.0 mg/kg BP897 group. Because differences in water control values existed between the treatment groups, an ANCOVA was conducted to control for differences in predrug baseline levels of activity. The ANCOVA indicated there was a main effect of BP897 treatment [$F(2,20) = 11.45$, $P \leq .05$] and a morphine dose by BP897 treatment interaction [$F(10,100) = 5.28$, $P \leq .05$] such that a dose of 0.3 mg/kg BP897 attenuated the effects of 200 and 300 mg/kg morphine and a dose of 1.0 mg/kg BP897 attenuated the effects of 30–300 mg/kg morphine (right panel).

### 3.3. Effects of caffeine alone and in combination with quinelorane, quinpirole, eticlopride, nafadotride and BP897

To determine the specificity of the modulatory actions of the DA D2/3 agonists and antagonists, the largest dose for each DA D2/3 agonist or antagonist tested in combination with morphine was examined for its ability to alter the locomotor actions of caffeine. Fig. 3 shows the effects of caffeine alone on total distance traveled and in combination with quinelorane and quinpirole (left panel) as well as in combination with eticlopride, nafadotride and BP897 (right panel). Caffeine produced a biphasic effect on total distance traveled. There was a main effect of caffeine dose [$F(4,28) = 13.33$, $P \leq .05$] such that doses of 10 and 30 mg/kg caffeine produced increases in total distance traveled of 64% and 30% relative to water control, respectively, whereas, a dose of 100 mg/kg caffeine significantly reduced

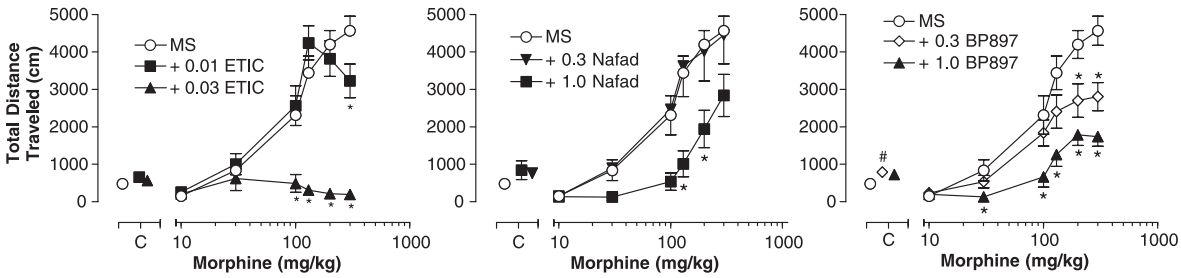


Fig. 2. Effects of morphine alone (MS, $n = 8$) and in combination with eticlopride (ETIC, $n = 8$) (left panel), nafadotride (Nafad, $n = 8$) (middle panel) and BP897 ($n = 8$) (right panel) on total distance traveled (cm). Data points above "C" represent the total distance traveled following water administration during the second 15-min component. Following the second component, mice received either (1) a morphine injection (10 mg/kg) or (2) an injection of morphine (10 mg/kg) in combination with a dose of one of an antagonist at the beginning of the third component. For the remaining components all mice received morphine injections. The morphine curve represented in each panel is the same. Each data point represents the mean total distance traveled during each 15-min component. Brackets indicate S.E.M. Where brackets are not visible, the S.E.M. fell within the data point. * Significant difference compared to morphine alone. #Significant difference compared to the water control of the morphine dose–effect curve.

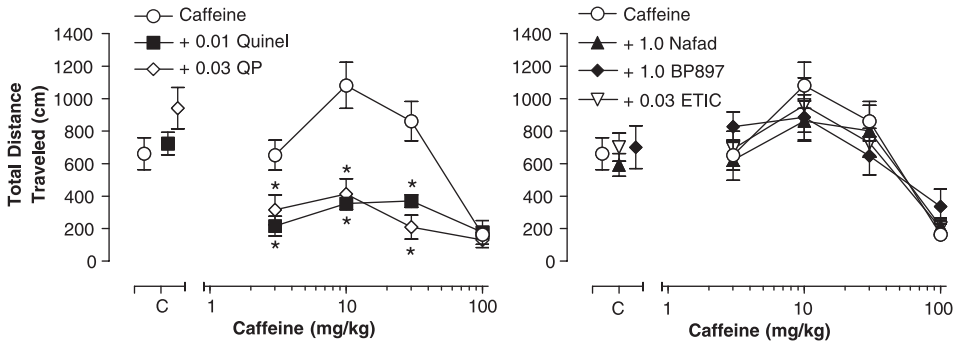


Fig. 3. Effects of caffeine alone ($n=8$) and in combination with quinelorane (Quinel, $n=8$) or quinpirole (QP, $n=8$) (left panel) and in combination with nafadotride (Nafad, $n=8$), BP897 ($n=8$) or eticlopride (ETIC, $n=8$) (right panel) on total distance traveled (cm). Data points above "C" represent the total distance traveled following water administration during the second 15-min component. Following the second component, mice received either (1) a caffeine injection (3.0 mg/kg) or (2) an injection of caffeine (3.0 mg/kg) in combination with a dose of an agonist (or antagonist) at the beginning of the third component. For the remaining components all mice received caffeine injections. The caffeine curve represented in each panel is the same. Each data point represents the mean total distance traveled during each 15-min component. Brackets indicate S.E.M. Where brackets are not visible, the S.E.M. fell within the data point. * Significant difference compared to caffeine alone.

total distance traveled relative to water control. The water control value obtained during the second component for the caffeine alone group did not differ from the water control values for the quinelorane [$t(14)=0.52$, $P>.05$], quinpirole [$t(14)=1.75$, $P>.05$], nafadotride [$t(14)=0.58$, $P>.05$], eticlopride [$t(14)=0.24$, $P>.05$] or BP897 [$t(14)=0.31$, $P>.05$] groups. An ANOVA indicated that there was an effect of quinelorane treatment [$F(1,14)=25.04$, $P\leq.05$] as well as a caffeine dose by quinelorane interaction [$F(3,42)=10.52$, $P\leq.05$] such that 0.01 mg/kg quinelorane significantly attenuated the effects of 3.0–30 mg/kg caffeine on total distance traveled. An ANOVA indicated that there was an effect of quinpirole treatment [$F(1,14)=18.65$, $P\leq.05$] as well as a caffeine dose by quinpirole interaction [$F(3,42)=8.82$, $P\leq.05$] such that 0.03 mg/kg quinpirole significantly attenuated the effects of 3.0–30 mg/kg caffeine on total distance traveled. An ANOVA indicated that there was no effect of nafadotride (1.0 mg/kg) [$F(1,14)=0.27$, $P>.05$], eticlopride (0.03 mg/kg) [$F(1,14)=0.14$, $P>.05$] or BP897 (1.0 mg/kg) [$F(1,14)=0.02$, $P>.05$] treatment on the total distance traveled induced by caffeine.

### 3.4. Effects of water alone and in combination with quinelorane, quinpirole, eticlopride, nafadotride and BP897

Fig. 4 shows the effects of water alone on total distance traveled and in combination with quinelorane and quinpirole (left panel) as well as in combination with eticlopride, nafadotride and BP897 (right panel). Water was repeatedly administered for five components following the initial water baseline control administered during the second component. The largest dose for each DA D2/3 agonist or antagonist tested in combination with morphine was tested alone to determine its effects relative to the water control group. The water control value obtained during the second component for the water-alone group did not differ from the water control values for the quinelorane [$t(14)=0.99$, $P>.05$] or quinpirole [$t(14)=0.75$, $P>.05$] groups. An ANOVA indicated that there was no effect of quinelorane (0.01 mg/kg) treatment [$F(1,14)=3.86$, $P>.05$], however, the P value approached significance at 0.07 (left panel). An ANOVA indicated that there was an effect of quinpirole (0.03 mg/kg) treatment [$F(1,14)=6.22$, $P\leq.05$] such that activity was

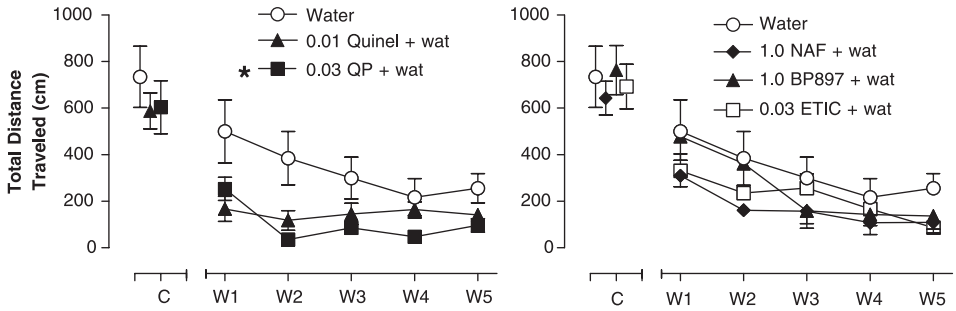


Fig. 4. Effects of water alone ($n=8$) and in combination with quinelorane (Quinel, $n=8$) or quinpirole (QP, $n=8$) (left panel) and in combination with nafadotride (Nafad, $n=8$), BP897 ($n=8$) or eticlopride (ETIC, $n=8$) (right panel) on total distance traveled (cm). Data points above "C" represent the total distance traveled following water administration during the second 15-min component. At the beginning of W1, mice received either (1) a water injection or (2) a drug injection. For the remaining components (W2–W5) all mice received water injections. The water-alone curve represented in each panel is the same. Each data point represents the mean total distance traveled during each 15-min component. Brackets indicate S.E.M. Where brackets are not visible, the S.E.M. fell within the data point. * Indicates a significant main effect of quinpirole treatment relative to water control.

reduced relative to the water-alone group (left panel). The water control value obtained during the second component for the water-alone group did not differ from the water control values for the nafadotride [$t(14)=0.61$, $P>.05$], eticlopride [$t(14)=0.26$, $P>.05$] or BP897 [$t(14)=0.17$, $P>.05$] groups. An ANOVA indicated that there was no effect of nafadotride (1.0 mg/kg) [$F(1,14)=2.86$, $P>.05$], eticlopride (0.03 mg/kg) [$F(1,14)=1.51$, $P>.05$] or BP897 (1.0 mg/kg) [$F(1,14)=0.53$, $P>.05$] treatment, confirming that these drugs had no effect on total distance traveled by themselves.

## 4. Discussion

The results of the present investigation indicate that morphine produces dose-dependent increases in locomotion when administered in a cumulatively dosed manner in male Swiss–Webster mice. At the highest dose tested (300 mg/kg), total distance traveled was increased by approximately 1000% relative to vehicle control activity levels. This cumulative dosing procedure is unique in that multiple doses of morphine can be tested in each mouse, which contrasts with conventional methods in which separate groups of mice are tested with different doses of morphine alone (e.g., Longoni et al., 1987; Rodriguez-Arias et al., 2000). Moreover, the modulatory actions of a single dose of a drug (e.g., D2/3 agonist or antagonist) can be determined in combination with multiple doses of morphine in an individual mouse. This ultimately results in dramatically reducing the total number of mice needed to complete a study.

Both the D2/3 antagonists eticlopride and nafadotride attenuated morphine-induced locomotion, although the magnitude of the attenuation obtained with nafadotride was not as great as that obtained with eticlopride. The partial D2/3 agonist BP897 dose-dependently reduced morphine-induced locomotion and produced intermediate levels of attenuation compared with eticlopride and nafadotride. Importantly, this attenuation occurred at doses of eticlopride, nafadotride and BP897 that did not alter activity relative to water control activity levels. Both the D2/3 agonists quinelorane and quinpirole produced a nonsignificant attenuation of morphine-induced locomotion at doses that either significantly (0.3 mg/kg quinpirole) or nonsignificantly (0.01 mg/kg quinelorane) reduced activity levels relative to water control activity levels. These results demonstrating that both antagonists and, albeit less robustly, agonists at D2/3 receptors, attenuate morphine-induced locomotion are similar to the findings that nafadotride, eticlopride, quinelorane and quinpirole attenuate mu opioid-like discriminative stimulus effects in rats (unpublished observations; Cook and Beardsley, 2001). The attenuation is likely the result of the agonists' ability to decrease DAergic activity through presynaptic receptors (Robertson et al., 1993; See et al., 1991) or a negative feedback pathway following postsynaptic receptor activation (Koeltzow et al., 1998; Timmerman et al., 1990) as well as the antagonists' ability to block DAergic activity via postsynaptic D2/3 receptor blockade.

The methodologies employed in the present study were designed to minimize habituation by exposing the mice to the test chambers for only 30 min (15-min nondrug period plus 15-min water period) before drug administration. This conservative approach accomplished the goal of producing moderate levels of non-drug-affected activity such that when the D2/3 agonists and antagonists were administered alone detection of suppressant effects could be observed. The attenuation of morphine-induced locomotion by quinpirole and quinelorane is likely the combined result of these agonists suppressing activity alone, which counteracted the increases produced by morphine, as well as a result of their ability to modulate DAergic activity following morphine administration (Kamei and Saitoh, 1996). The attenuation produced by eticlopride, nafadotride and BP897 appears to be the direct result of a pharmacological interaction between D2/3 receptor activity and the blockade of the increased DAergic activity produced by morphine.

The present results concur with previous studies in which the D2/3 antagonists sulpiride and raclopride were reported to attenuate the locomotor-activating effects of morphine (Rodriguez-Arias et al., 2000; Zarrindast and Zarghi, 1992). In the Rodriguez-Arias et al. (2000) study, however, raclopride reduced morphine-induced locomotion at doses that by themselves suppressed activity suggesting that this attenuation was nonspecific in nature. Additionally, in the Zarrindast and Zarghi (1992) study, the effect of sulpiride alone on nondrug levels of activity was not reported, although the sulpiride dose tested (25 mg/kg) had been previously identified as a dose that can reduce baseline levels of activity (Chausmer and Katz, 2001).

The marginal attenuation of morphine's effects obtained with quinelorane and quinpirole in the present study is similar to the results of a previous study in which quinpirole (0.5 mg/kg) produced approximately a 10% reduction in morphine-induced (10 mg/kg) locomotion (Zarrindast and Zarghi, 1992). Interestingly, quinpirole produced increases in locomotion that were similar to that obtained with morphine alone (10 mg/kg) (Zarrindast and Zarghi, 1992). In contrast with the results obtained with the D2/3 agonists in the present study, the D2/3 agonist 7-OH-DPAT attenuated the locomotor-activating effects of morphine in mice at doses that did not markedly alter nondrug levels of activity when administered alone (Suzuki et al., 1995). Morphine-induced locomotion (10 and 20 mg/kg) was reduced by approximately 75% by 7-OH-DPAT (Suzuki et al., 1995), whereas the maximal attenuation obtained with quinpirole and quinelorane in the present study was approximately 37% and 50%, respectively, at the 300 mg/kg morphine dose. The discrepancies in the magnitude of the attenuation between the present findings and those previously reported

may be the result of procedural differences as well as differences in the strain of mice tested.

Several of the behavioral effects induced by caffeine, including increased locomotion, are believed to be mediated, in part, through the DA system (Garrett and Griffiths, 1997). As such, the highest doses of the D2/3 agonists and antagonists tested in combination with morphine were examined in combination with caffeine. Caffeine produced a biphasic effect on total distance traveled with peak stimulatory effects occurring at a dose of 10 mg/kg and inhibitory effects occurring at 100 mg/kg. This biphasic action by caffeine is similar to the findings of other studies using mice (Kaplan et al., 1992; Logan et al., 1986) and rats (Garrett and Holtzman, 1994; White et al., 1978). Both quinpirole and quinelorane significantly attenuated the locomotor stimulant effects of caffeine, whereas nafadotride, eticlopride and BP897 had no effect on caffeine-induced locomotion. It is unclear, however, as to how much of the attenuation produced by quinelorane and quinpirole was the direct result of these drugs suppressing activity alone. The failure of eticlopride to alter the effects of caffeine contrasts with a study in which both eticlopride and sulpiride reduce caffeine-induced locomotion (Garrett and Holtzman, 1994). Although the authors report that eticlopride had little effect on activity levels alone, the rats received 5 days of habituation to the test chambers before testing and on the day of testing each rat received an additional 15 min of habituation. Baseline levels of nondrug activity may have been so low that detection of further decreases by eticlopride may have been precluded. Thus, the lack of effect of eticlopride in the present investigation compared to the Garrett and Holtzman (1994) study may be, in part, related to the differing amounts of habituation between the studies. The D2/3 antagonist pimozide attenuates the stimulant effects of caffeine in mice, but this attenuation is obtained at doses that significantly decrease activity alone in nonhabituated mice (Estler, 1979). In contrast with studies employing extensive habituation periods in which the resulting baseline levels of activity are near zero, in the present study the implementation of a brief "habituation" period consisting of a 15-min nondrugged period followed by a 15-min water control period resulted in moderate levels of baseline activity and, thus, likely provides a bidirectional baseline against which to measure the modulatory role of D2/3 receptors. As such, the present results with the D2/3 antagonists suggest that D2/3 receptors are not intrinsically involved in the locomotor effects of caffeine. The results with quinelorane and quinpirole suggest that D2/3 receptor activation modulates caffeine-induced locomotion. However, the resulting levels of activity produced by caffeine in combination with a D2/3 agonist (200–400 cm traveled, Fig. 3) are similar to the activity levels obtained with these D2/3 agonists alone (~200 cm traveled, Fig. 4), which suggests that a nonspecific modulatory role cannot be excluded. Furthermore, that doses of the D2/3 antagonists, which when administered alone did not alter activity, failed to alter caffeine-induced locomotion, implies that the attenuation of caffeine-induced locomotion by D2/3 antagonists observed in previous studies may, in part, be nonspecific in nature.

All of the DA antagonists tested exhibit affinity for both D2 and D3 receptors, but with different degrees of selectivity. For example, nafadotride exhibits approximately 10-fold selectivity for D3 over D2 receptors (Audinot et al., 1998; Sautel et al., 1995), whereas the selectivity of eticlopride for D2 over D3 receptors approaches 15-fold (Levant, 1997). The greater attenuation obtained with eticlopride, a preferential D2 receptor antagonist, relative to nafadotride, a D3-receptor-preferring antagonist, suggests that D2 receptors, more so than D3 receptors, are involved in modulating the locomotor-activating effects of mu opioids. A modulatory role for the D3 receptor, however, cannot be eliminated, as nafadotride doses equal to, or smaller than 1.0 mg/kg sc (as in the present study), exhibit negligible binding at D2 receptors (Levant and Vansell, 1997). To maintain selectivity for D3 over D2 receptors, doses larger than 1.0 mg/kg nafadotride were not tested. The partial agonist BP897 exhibits agonist and antagonist activity at D3 receptors and only antagonist properties at D2 receptors (Pilla et al., 1999; Wicke and Garcia-Ladona, 2001; Wood et al., 2000). The attenuation produced by BP897 was greater than that observed with nafadotride, but less than that obtained with eticlopride. Taken together, these results suggest that the antagonist actions of BP897 at D2 more so than D3 receptors are likely mediating the attenuation observed against morphine.

As with the DA antagonists, both quinelorane and quinpirole bind to both D2 and D3 receptors. In binding assays, quinelorane and quinpirole exhibit greater selectivity for D3 than D2 receptors (see Levant, 1997). Results from functional assays indicate that quinpirole exhibits marginal selectivity for D3 over D2 receptors, whereas quinelorane remains selective for D3 receptors (Chio et al., 1994; Sautel et al., 1995). There is evidence that quinpirole differs from other D2/3 agonists in that it binds to a potential novel binding site (Gilliland et al., 2000; Levant et al., 1996). This novel site of action may partially explain why quinpirole can produce behavioral effects that differ from other classical D2/3 agonists under some circumstances (Cook et al., 1999, 2000; Cory-Slechta et al., 1996; Depoortere et al., 1996). The observation that quinpirole and quinelorane produced similar effects in the present study suggests that this novel mechanism of action is not likely involved in its locomotor actions when administered alone or in combination with morphine or caffeine.

The cumulative dosing procedure employed in the present study was extremely sensitive to not only the stimulant, but also the inhibitory properties of drugs, and appears well suited to study complex drug–drug interactions. However, this locomotor procedure is not without its limitations. Firstly, the resulting activity levels obtained for each dose of morphine (15 min post administration) in the present

study do not correspond to the maximal activity levels for each dose, as peak activity levels with morphine occur approximately 60 min post administration (e.g., Kuribara, 1995). Secondly, the current experimental design does not take into account pharmacokinetic differences across D2/3 drugs. All of the D2/3 agonists and antagonists were coadministered with the first morphine dose following the water baseline component. Therefore, it is possible that the peak effects of each D2/3 drug occurred at differing time points across the 1- to 1.5-h test session. It is unclear how and if differing pharmacokinetic profiles may have influenced the attenuation obtained in the present study.

The results of the present study demonstrate that doses of D2/3 antagonists, which attenuate the locomotor-activating effects of morphine, fail to modify the locomotor-activating effects of caffeine. This suggests that although both mu opioids and methylxanthines indirectly alter DAergic activity, the manner in which the DA system interacts with the opioid and methylxanthine systems is not the same. In fact, there are data suggesting that mu opioid- and caffeine-induced locomotion occur independent of presynaptic DA release (Joyce and Koob, 1981; Vaccarino et al., 1986) and that the locomotor-activating effects of mu opioids and caffeine are mediated by distinct neural mechanisms. For example, reports that the administration of the $GABA_A$ agonist muscimol into the ventral pallidum blocks the locomotor response to heroin, but not to caffeine, suggests that mu opioids stimulate locomotion through inhibition of GABAergic activity and that caffeine produces locomotion through a GABA-independent neural substrate (Swerdlow and Koob, 1985). Thus, the failure of the D2/3 antagonists to attenuate caffeine-induced locomotion suggests that DAergic activity produced by caffeine is not an integral part of caffeine's locomotor-activating effects. In contrast, the D2/3 receptor antagonism results demonstrate that the DA system is intimately involved in mediating the locomotion induced by morphine.

## Acknowledgements

This work was supported by an Institutional Training Grant from the National Institute on Drug Abuse (DA07027). CDC was supported by a National Institute on Drug Abuse Training Grant (DA07027). The authors acknowledge the technical assistance provided by Laura Biddlestone.

## References

Acquas E, Tanda G, Di Chiara G. Differential effects of caffeine on dopamine and acetylcholine transmission in brain areas of drug-naive and caffeine-pretreated rats. Neuropsychopharmacology 2002;27:182–93.

Audinot V, Newman-Tancredi A, Gobert A, Rivet JM, Brocco M, Lejeune F, et al. A comparative in vitro and in vivo pharmacological characterization of the novel dopamine D3 receptor antagonists (+)-S 14297, nafadotride, GR 103,691 and U 99194. J Pharmacol Exp Ther 1998; 287:187–97.

Brase DA, Loh HH, Way EL. Comparison of the effects of morphine on locomotor activity, analgesia and primary and protracted physical dependence in six mouse strains. J Pharmacol Exp Ther 1977;201: 368–74.

Chausmer AL, Katz JL. The role of $D_2$-like dopamine receptors in the locomotor stimulant effects of cocaine alone in mice. Psychopharmacology 2001;155:69–77.

Chio CL, Lajiness ME, Huff RM. Activation of heterologously expressed D3 dopamine receptors: comparison with D2 dopamine receptors. Mol Pharmacol 1994;45:51–60.

Colpaert FC, Niemegeers JE, Janssen PA. Differential haloperidol effect on two indices of fentanyl–saline discrimination. Psychopharmacology 1977;53:169–73.

Cook CD, Beardsley PM. Effects of dopamine D3/2 drugs on the discriminative stimulus effects of mu opioids in heroin-trained rats. Drug Alcohol Depend 2001;63:S31.

Cook CD, Picker MJ. Dopaminergic activity and the discriminative stimulus effects of mu opioids in pigeons: importance of training dose and attenuation by the D3 agonist (±)-7-OH-DPAT. Psychopharmacology 1998;136:59–69.

Cook CD, Rodefer JS, Picker MJ. Selective attenuation of the antinociceptive effects of mu opioid by the putative dopamine D3 agonist 7-OH-DPAT. Psychopharmacology 1999;144:239–47.

Cook CD, Barrett AC, Syvanthong C, Picker MJ. Modulatory effects of dopamine D3/2 agonists on kappa opioid-induced antinociception and diuresis in the rat. Psychopharmacology 2000;152:14–23.

Corrigall WA, Coen KM. Selective D 1 and D 2 dopamine antagonists decrease response rates of food-maintained behavior and reduce the discriminative stimulus produced by heroin. Pharmacol Biochem Behav 1990;35:351–5.

Cory-Slechta DA, Zuch CL, Fox RAV. Comparison of a pre- vs. a putative postsynaptic dose of quinpirole. Pharmacol Biochem Behav 1996;55: 423–33.

Daly JW, Bruns RF, Snyder SH. Adenosine receptors in the central nervous system: relationship to the central actions of methylxanthines. Life Sci 1981;28:2083–97.

David V, Durkin TP, Cazala P. Differential effects of the dopamine D2/D3 receptor antagonist sulpiride on self-administration of morphine into the ventral tegmental area or the nucleus accumbens. Psychopharmacology 2002;160:307–17.

Depoortere R, Perrault G, Sanger DJ. Behavioral effects in the rat of the putative dopamine D3 receptor agonist 7-OH-DPAT: comparison with quinpirole and apomorphine. Psychopharmacology 1996;124:231–40.

Di Chiara G, Imperato A. Opposite effects of mu and kappa opiate agonists on dopamine release in the nucleus accumbens and in the dorsal caudate of freely moving rats. J Pharmacol Exp Ther 1988;244:1067–80.

Estler CJ. Influence of pimozide on the locomotor hyperactivity produced by caffeine. J Pharm Pharmacol 1979;31:126–7.

Fujiwara H. Comparative studies of sulpiride and classical neuroleptics on induction of catalepsy, locomotor activity, and brain dopamine metabolism in mice. Pharmacol Biochem Behav 1992;41:301–8.

Garrett BE, Griffiths RR. The role of dopamine in the behavioral effects of caffeine in animals and humans. Pharmacol Biochem Behav 1997;57: 533–41.

Garrett BE, Holtzman SG. D1 and D2 dopamine receptor antagonists block caffeine-induced stimulation of locomotor activity in rats. Pharmacol Biochem Behav 1994;47:89–94.

Gerrits M, Ramsey NF, Wolterink G, van Ree JM. Lack of evidence for an involvement of nucleus accumbens dopamine D1 receptors in the initiation of heroin self-administration in the rat. Psychopharmacology 1994;114:486–94.

Gilliland SL, Alper RH, Levant B. Pharmacology of quinpirole-stimulated [35S]GTPgammaS binding: discrepancy with receptor binding profile. Eur J Pharmacol 2000;392:125–8.

Hemby SE, Smith JE, Dworkin SI. The effects of eticlopride and naltrexone

on responding maintained by food, cocaine, heroin and cocaine/heroin combinations in rats. J Pharmacol Exp Ther 1996;277:1247–58.
Joyce EM, Koob GF. Amphetamine-, scopolamine- and caffeine-induced locomotor activity following 6-hydroxydopamine lesions of the mesolimbic dopamine system. Psychopharmacology 1981;73:311–3.
Kamei J, Saitoh A. Modification of the effects of 7-OH-DPAT, a dopamine D3-receptor agonist, on morphine-induced hyperlocomotion by diabetes. Life Sci 1996;59:773–80.
Kaplan GB, Greenblatt DJ, Kent MA, Cotreau MM, Arcelin G, Shader RI. Caffeine-induced behavioral stimulation is dose-dependent and associated with A1 adenosine receptor occupancy. Neuropsychopharmacology 1992;6:145–53.
Koeltzow TE, Xu M, Cooper DC, Hu X-T, Tonegawa S, Wolf ME, et al. Alterations in dopamine release but not dopamine autoreceptor function in dopamine D3 receptor mutant mice. J Neurosci 1998;18:2231–8.
Kuribara H. Modification of morphine sensitization by opioid and dopamine receptor antagonists: evaluation by studying ambulation in mice. Eur J Pharmacol 1995;275:251–8.
Kuschinsky K, Hornykiewicz O. Effects of morphine on striatal dopamine metabolism: possible mechanism of its opposite effect on locomotor activity in rats and mice. Eur J Pharmacol 1974;26:41–50.
Levant B. The D3 dopamine receptor: neurobiology and potential clinical relevance. Pharmacol Rev 1997;49:231–52.
Levant B, Vansell NR. In vivo occupancy of $D_2$ dopamine receptors by nafadotride. Neuropsychopharmacology 1997;17:67–71.
Levant B, Moehlenkamp JD, Morgan KA, Leonard NL, Cheng CC. Modulation of [3H]quinpirole binding in brain by monoamine oxidase inhibitors: evidence for a potential novel binding site. J Pharmacol Exp Ther 1996;278:145–53.
Logan L, Seale TW, Carney JM. Inherent differences in sensitivity to methylxanthines among inbred mice. Pharmacol Biochem Behav 1986;24:1281–6.
Longoni R, Spina L, Di Chiara G. Dopaminergic D-1 receptors: essential role in morphine-induced hypermotility. Psychopharmacology 1987;93:401–2.
Manzanedo C, Aguilar MA, Minarro J. The effects of dopamine D2 and D3 antagonists on spontaneous motor activity and morphine-induced hyperactivity in male mice. Psychopharmacology 1999;143:82–8.
McCarten MD, Lal H. Attenuation of the discriminative stimulus strength of morphine by haloperidol. Neuropharmacology 1979;18:465–7.
Pilla M, Perachon S, Sautel F, Garrido F, Mann A, Wermuth CG, et al. Selective inhibition of cocaine-seeking behaviour by a partial dopamine D3 receptor agonist. Nature 1999;400:371–5.
Robertson GS, Tham CS, Wilson C, Jakubovic A, Fibiger HC. In vivo comparisons of the effects of quinpirole and the putative presynaptic dopaminergic agonists B-HT 920 and SND 919 on striatal dopamine and acetylcholine release. J Pharmacol Exp Ther 1993;264:1344–51.
Rodriguez-Arias M, Broseta I, Aguilar MA, Minarro J. Lack of specific effects of selective D(1) and D(2) dopamine antagonists vs. risperidone on morphine-induced hyperactivity. Pharmacol Biochem Behav 2000;66:189–97.
Sautel F, Griffon N, Levesque D, Pilon C, Schwartz JC, Sokoloff P. A functional test identifies dopamine agonists selective for D3 versus D2 receptors. NeuroReport 1995;6:329–32.
See RE, Sorg BA, Chapman MA, Kalivas PW. In vivo assessment of release and metabolism of dopamine in the ventrolateral striatum of awake rats following administration of dopamine D1 and D2 receptor agonists and antagonists. Neuropharmacology 1991;30:1269–74.
Shippenberg TS, Herz A. Motivational effects of opioids: influence of D-1 versus D-2 receptor antagonists. Eur J Pharmacol 1988;151:233–42.
Shippenberg TS, Bals-Kubik R, Herz A. Examination of the neurochemical substrates mediating the motivational effects of opioids: role of the mesolimbic dopamine system and D-1 vs. D-2 dopamine receptors. J Pharmacol Exp Ther 1993;265:53–9.
Snyder SH, Katims JJ, Annau A, Bruns RF, Daly JW. Adenosine receptors and the behavioral effects of methylxanthines. Proc Natl Acad Sci U S A 1981;78:3260–4.
Suzuki T, Maeda J, Funada M, Misawa M. The D3-receptor agonist (±)-7-hydroxy-*N*,*N*-di-*n*-propyl-2-aminotetralin (7-OH-DPAT) attenuates morphine-induced hyperlocomotion in mice. Neurosci Lett 1995;187:45–8.
Swerdlow NR, Koob GF. Separate neural substrates of the locomotor-activating properties of amphetamine, heroin, caffeine and corticotropin releasing factor (CRF) in the rat. Pharmacol Biochem Behav 1985;23:303–7.
Timmerman W, De Vries JB, Westerink BH. Effects of D-2 agonists on the release of dopamine: localization of the mechanism of action. Naunyn Schmiedebergs Arch Pharmacol 1990;342:650–4.
Ukai M, Hiraiwa E, Kameyama T. Pentazocine-like discriminative stimulus effects of morphine are blocked by the dopamine D-1 antagonist SCH 23390, but not by the dopamine D-2 antagonist sulpiride. Brain Res 1991;541:146–8.
Vaccarino FJ, Amalric M, Swerdlow NR, Koob GF. Blockade of amphetamine but not opiate-induced locomotion following antagonism of dopamine function in the rat. Pharmacol Biochem Behav 1986;24:61–5.
White BC, Simpson CC, Adams JE, Harkins Jr D. Monoamine synthesis and caffeine-induced locomotor activity. Neuropharmacology 1978;17:511–3.
Wicke K, Garcia-Ladona J. The dopamine D3 receptor partial agonist, BP 897, is an antagonist at human dopamine D3 receptors and at rat somatodendritic dopamine D3 receptors. Eur J Pharmacol 2001;424:85–90.
Winger G, France CP, Woods JH, Smith CB, Medzihradsky F, Iorio MA. Opioid and non-opioid effects of novel butyrophenone analogues. Neuropsychopharmacology 1992;7:177–87.
Wise RA, Leone P, Rivest R, Leeb K. Elevations of nucleus accumbens dopamine and DOPAC levels during intravenous heroin self-administration. Synapse 1995;21:140–8.
Wood PL, McQuade P, Richard JW, Thakur M. Agonist/antagonist analgesics and nigrostiatal dopamine metabolism in the rat: evidence of receptor dualism. Life Sci 1983;33:759–62.
Wood MD, Boyfield I, Nash DJ, Jewitt FR, Avenell KY, Riley GJ. Evidence for antagonist activity of the dopamine D3 receptor partial agonist, BP 897, at human dopamine D3 receptor. Eur J Pharmacol 2000;407:47–51.
Zarrindast MR, Zarghi A. Morphine stimulates locomotor activity by an indirect dopaminergic mechanism: possible D-1 and D-2 receptor involvement. Gen Pharmacol 1992;23:1221–5.