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Short communication

Fluoxetine attenuates morphine-induced locomotion and blocks morphine-sensitization

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Abstract

Repeated morphine treatments result in sensitization, an increase in the efficacy of morphine to stimulate locomotor activity. This study examined the effects of increasing serotonin (5-hydroxytryptamine, 5-HT) transmission on morphine-sensitization. For five days rats were administered saline or 5.0 mg/kg fluoxetine prior to treatment with saline or 5.0 mg/kg morphine. Twenty-one days later, rats were tested for their locomotor response to 2.0 mg/kg morphine. Fluoxetine treatment attenuated the locomotor activating effect of acute morphine treatments and blocked the sensitized response to the morphine challenge. These results indicate that increased 5-HT transmission attenuates the locomotor stimulating effects of morphine and prevents the development of morphine-sensitization. © 1997 Elsevier Science B.V.

Keywords: 5-HT (5-hydroxytryptamine, serotonin); Locomotor activity; (Rat); Serotonin reuptake inhibitor, selective

1. Introduction

Morphine stimulates locomotor activity when administered in low to moderate doses (Babbini and Davis, 1972) and this effect exhibits sensitization following repeated treatments, such that morphine becomes more efficacious in enhancing locomotor activity (Babbini and Davis, 1972; Kalivas and Duffy, 1987). The locomotor activating effect of acute and repeated morphine treatments is mediated, in part, by activation of the mesolimbic dopamine system (Joyce and Iversen, 1979; Vezina et al., 1987; Pei et al., 1993; Spanagel et al., 1993).

In addition to stimulating mesolimbic dopamine release, systemic morphine treatments have been shown to induce increases in serotonin (5-hydroxytryptamine, 5-HT) release in regions of the brain that receive projections from the dorsal raphe nucleus, including the mesolimbic dopamine system (Broderick, 1985a,b; Tao and Auerbach, 1994). Local infusion of morphine into the dorsal raphe nucleus, but not the median raphe, also increases 5-HT in the nucleus accumbens, the terminal region of the mesolimbic dopamine system (Tao and Auerbach, 1994). Together, these findings raise the possibility that 5-HT mechanisms contribute to the locomotor activating effects of acute and repeated morphine treatments.

The present study examined whether enhancing 5-HT transmission would alter the development and expression of behavioral sensitization induced by repeated morphine treatments. To this end, the effects of the selective serotonin reuptake inhibitor fluoxetine on the locomotor activating effect of acute and repeated morphine treatments were assessed.

2. Materials and methods

Twenty-six Male Wistar rats (Charles River, Canada) weighing 250–275 g at the start of the experiment, were housed in individual hanging wire mesh cages in a temperature and light controlled environment, with lights on–off at 07.00–19.00 h. Rats had ad libitum access to water and standard Purina lab chow pellets throughout the experiment.

2.1. Drugs

Morphine (Health and Welfare, Canada) was dissolved in saline vehicle. Fluoxetine (Eli Lilly) was dissolved in

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distilled water vehicle. All drugs were administered intraperitoneally, in a volume of 1 ml/kg.

2.2. Apparatus

To measure locomotion, 16 photocell beam cages housed in another room were utilized. The cages measured 34 cm × 33 cm, with two photocell beams placed 3 cm above the floor, with one beam located 11 cm from the front of the cage and the other beam located 11 cm from the back of the cage. The floor and front wall of the cages were constructed out of wire mesh, with the sides and back wall constructed out of metal. The cages were interfaced with an IBM compatible computer that recorded photocell beam interruptions as counts.

2.3. Training phase

Animals were habituated to the locomotor activity cages for three h (15.00–18.00 h) on each of two days prior to drug testing. On the day following the last habituation day, and on each of the next five consecutive days, animals were tested for their locomotor response to drug treatments. At the beginning of each test session, animals were habituated to the activity cages for 30 min. Following this 30 min habituation period, rats were injected with either vehicle (distilled water) or fluoxetine (5 mg/kg) and placed into the activity chambers for another 30 min. Subsequently, animals were injected with either saline or morphine (5 mg/kg) and placed into the activity cages for a further 3 h. This design yielded four groups: vehicle–saline ($n = 6$), vehicle–morphine ($n = 7$), fluoxetine–saline ($n = 7$), fluoxetine–morphine ($n = 6$). Animals were randomly assigned to one of the four experimental conditions.

2.4. Morphine test day

Twenty-one days following the last training session, animals were habituated to the locomotor activity cages for one hour. Subsequently, animals were injected with 2.0 mg/kg morphine and activity was measured for 3 h.

2.5. Statistics

Data were analyzed using analysis of variance (ANOVA), with post-hoc analyses carried out using Least Significant Difference test.

3. Results

3.1. Training phase

A three-way ANOVA, with condition as the between-subjects factor and day and interval as the within-subjects factors, revealed a significant interaction between condition and interval, $F(6,44) = 3.84$, $P < 0.05$. There were also significant main effects of condition, $F(3,22) = 9.08$, and interval, $F(2,44) = 55.07$, $P < 0.05$. As shown in Fig. 1, animals in the morphine condition exhibited significantly higher levels of locomotor activity than animals in the other conditions during the first and second h following morphine administration across all training days. In addition, animals in the fluoxetine–morphine condition exhibited significantly higher levels of locomotor activity

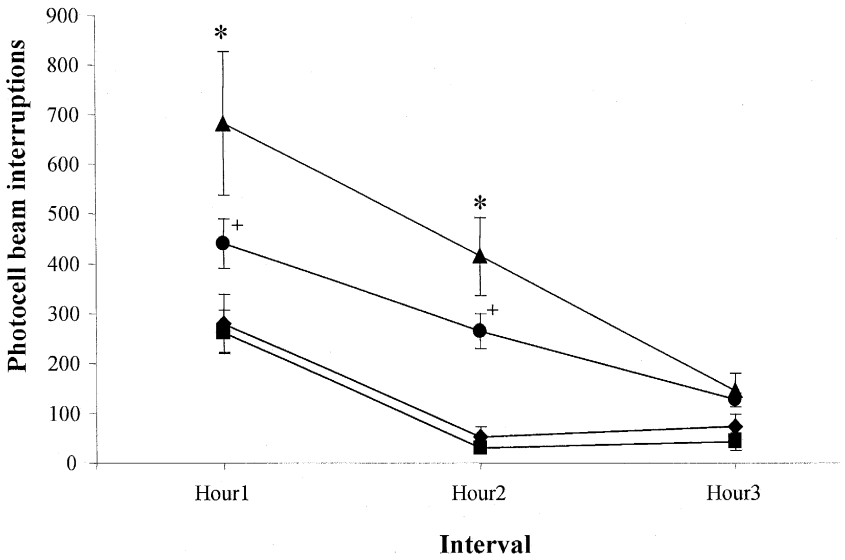

Fig. 1. Average (±S.E.M.) amount of daily locomotor activity across the 3-h test sessions exhibited by rats treated with either vehicle + saline (♦), 5 mg/kg fluoxetine + saline (■), vehicle + 5 mg/kg morphine (▲), or 5 mg/kg fluoxetine + 5 mg/kg morphine (●) across the five training days. * Significantly higher than vehicle–saline, fluoxetine–saline and fluoxetine–morphine treated animals; + significantly higher than vehicle + saline and fluoxetine + saline treated animals.

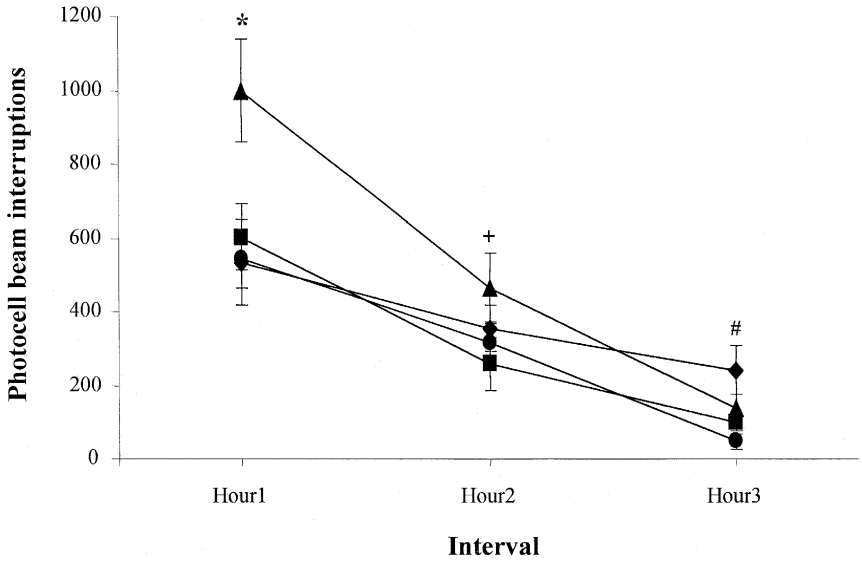

Fig. 2. Average (±S.E.M.) amount of locomotor activity exhibited by rats in response to a challenge dose of 2.0 mg/kg morphine administered 21 days following the last of 5 days of pretreatment with either vehicle + saline (♦), 5 mg/kg fluoxetine + saline (■), vehicle + 5 mg/kg morphine (▲), or 5 mg/kg fluoxetine + 5 mg/kg morphine (●). * Vehicle–morphine significantly higher than vehicle–saline, fluoxetine–saline and fluoxetine–morphine pretreatment groups; + vehicle–morphine significantly higher than vehicle–saline, and fluoxetine–saline pretreatment groups; # vehicle–saline significantly higher than fluoxetine–saline.

than animals in the saline and fluoxetine conditions during the first and second h following morphine administration across all training days.

3.2. Sensitization

A two-way ANOVA, with condition as the between-subjects factor and interval as the within-subjects factor, revealed a significant condition × interval interaction, $F(6,44) = 3.74$, $P < 0.05$. There was also a significant main effect of interval, $F(2,44) = 71.03$, $P < 0.05$. Fig. 2 shows that animals in the morphine condition exhibited significantly higher levels of locomotor activity than animals in all of the other groups in the first hour following morphine treatment. Animals in the morphine condition also exhibited significantly higher levels of locomotor activity than animals in the fluoxetine condition during the second hour following morphine treatment. In the third hour following morphine treatment, animals in the saline condition exhibited higher levels of locomotor activity when compared to animals in the fluoxetine condition.

4. Discussion

In the present study, animals administered 5.0 mg/kg morphine exhibited significantly higher levels of locomotor activity when compared to saline treated animals. Pretreatment with fluoxetine prior to morphine resulted in an attenuation of the locomotor activating effect of morphine. Rats administered fluoxetine prior to morphine exhibited significantly less locomotor activity than animals administered morphine, but significantly more locomotor activity than animals treated with saline. Fluoxetine, in and of itself, was without effect on locomotor activity. These results indicate that increases in 5-HT activity, via inhibition of 5-HT reuptake, attenuates the locomotor activating effect of morphine without affecting baseline locomotor activity.

There was no increase in locomotor activity across the five days of treatment with 5.0 mg/kg morphine. However, when challenged with a 2.0 mg/kg dose of morphine 21 days following the end of the training phase, rats that had received prior morphine treatments exhibited significantly higher levels of locomotor activity as compared to control rats. This sensitized behavioral response to the challenge dose of morphine was blocked by pretreating rats with fluoxetine prior to morphine treatment during the training sessions. This was evidenced by the fact that rats in fluoxetine–morphine condition exhibited significantly lower levels of locomotor activity compared to rats in the vehicle–morphine condition. Indeed, rats in the fluoxetine–morphine condition exhibited locomotor scores similar to those of rats that received vehicle-saline treatments during the training sessions.

The mechanism through which fluoxetine attenuates the locomotor activating effect of morphine is not known. Morphine stimulates locomotor activity, in part, by activating mesolimbic dopamine transmission (Joyce and Iversen, 1979; Vezina et al., 1987; Pei et al., 1993; Spanagel et al., 1993). Morphine also enhances 5-HT transmission (Broderick, 1985a,b) and citalopram, a selective 5-HT uptake blocker, enhanced the 5-HT-stimulatory effect of morphine (Tao and Auerbach, 1994). Although exogenously applied 5-HT has been shown to stimulate

mesolimbic DA transmission (Guan and McBride, 1989; Parsons and Justice, 1993), fenfluramine, a releaser of 5-HT, inhibited the stimulating effect of morphine on dopamine transmission in the nucleus accumbens, the terminal region of the mesolimbic dopamine system (Spampinato et al., 1984). Fenfluramine has also been shown to reduce heroin self-administration, an effect that is antagonized by the 5-$HT_{1/2}$ receptor antagonist metergoline (Higgins et al., 1994). Thus, fluoxetine may enhance the morphine-induced increase in 5-HT activity and this may function to inhibit opiate-induced dopamine activation and opiate-induced behaviors, possibly via an action of 5-HT at 5-$HT_{1/2}$ receptors.

There is good evidence that the ventral tegmental area, the cell body region of the mesolimbic dopamine system, is a critical locus for morphine-induced behavioral sensitization (Vezina et al., 1987; Kalivas and Duffy, 1987). Administration of the opioid antagonist naltrexone into the ventral tegmental area, but not nucleus accumbens, blocked the sensitized locomotor response to repeated systemic morphine treatments (Kalivas and Duffy, 1987). Further, repeated treatments with morphine or selective μ agonists into the ventral tegmental area (but not into the nucleus accumbens) resulted in behavioral sensitization (Vezina et al., 1987; Kalivas and Duffy, 1987). Together, these results indicate a role for ventral tegmental area μ-receptor stimulation in the development of sensitization, although κ-receptor stimulation has also been shown to modulate morphine-induced sensitization (Spanagel, 1995).

The ventral tegmental area contains a significant number of 5-HT neurons, and these make synaptic contact with both dopamine- and non-dopamine-containing cells (Herve et al., 1987). Further, there is evidence that 5-HT inhibits ventral tegmental area dopamine neurons in vivo, with a pharmacology suggesting an action at the 5-HT_{2C} receptor subtype (Prisco et al., 1994). Thus, one possibility is that acute fluoxetine treatment stimulates 5-HT activity, and potentiates morphine-induced 5-HT stimulation, in the ventral tegmental area. The net effect of fluoxetine treatment would be to increase 5-HT in the ventral tegmental area, which would serve to diminish mesolimbic dopamine transmission and thereby attenuate morphine-induced locomotion acutely and block the development of morphine-sensitization. It must be noted however, that recent evidence suggests that fluoxetine may function as a 5-HT_{2C} antagonist (Ni and Miledi, 1997), and it remains to be determined whether the 5-HT_{2C} receptor blocking property of fluoxetine is critical for the inhibition of morphine-locomotion produced by fluoxetine treatment.

The results of the present study indicate that 5-HT activity does not contribute to the expression of morphine-induced locomotion and may, in fact, be a limiting factor in the locomotor stimulatory effect of morphine. Evidence presented here suggests that increasing 5-HT activity attenuates the locomotor activating effect of acute morphine and blocks morphine-induced sensitization.

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