

Effects of Alprazolam and Fluoxetine on Morphine Sensitization in Mice

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Summary

Benzodiazepines seem to be frequently abused in conjunction with opioids. Fluoxetine was reported to block morphine locomotor sensitization in rats. Sensitization has been implicated in some aspects of drug abuse. We have investigated the effect of alprazolam (0.25 mg/kg) and fluoxetine (5 mg/kg) on the development and expression of sensitization to the locomotor stimulant effect of morphine (10 mg/kg) in mice. Sensitization was produced by daily injections of morphine (10 mg/kg) for 10 days. There was a clear sensitization of locomotor activity produced by morphine in photocell activity cages but co-administration of alprazolam with morphine had no effect on the degree of sensitization. Alprazolam was also without effect on the expression of the sensitized response to morphine in mice sensitized with morphine alone. Fluoxetine partly reduced both the development and expression of morphine sensitization. In conclusion, the present experiments have not yielded evidence that alprazolam may influence the development or the expression of sensitization to morphine. However, they have corroborated and extended results indicating that fluoxetine can attenuate, to a certain level, the development and expression of morphine sensitization.

Key words

Morphine • Alprazolam • Fluoxetine • Sensitization • Locomotion

Introduction

Opioid abuse is frequently associated with abuse of benzodiazepines (Darke *et al.* 1995, Gossop *et al.* 1998, Petry and Bickel 1998, Leri and Franklin 2000,). Clinical experience suggests that benzodiazepines have a relatively low liability for abuse, except as an adjunct to other drugs of abuse (Woods and Winger 1995). The reasons for this combined abuse are not well understood. Do benzodiazepines increase the reinforcing properties or abuse liability of opioids? No strong behavioral evidence for this possibility has been presented.

Sensitization to the effect of drugs has been implicated in several aspects of drug abuse, including the development of dependence, craving and relapse. Sensitization is characterized by a progressive augmentation of behavioral effects elicited by the repeated administration of drugs. While repeated administration of morphine at intervals shorter than 12 h may induce tolerance, the repeated administration with an interdose interval of 24 h elicited sensitization to the locomotor stimulant effect (Kuribara 1996). In experimental studies, sensitization was observed after repeated administration of opioids (Kumar *et al.* 1971, Babbini and Davis 1972), cocaine (Post and Rose 1976),

amphetamine (Segal and Mandell 1974, Robinson and Becker 1986), ethanol (Masur *et al.* 1986, Cunningham and Noble 1992) and nicotine (Ksir *et al.* 1985, Shoaib *et al.* 1994). Sensitization can last weeks to months after cessation of drug treatment (Schoffelmeer *et al.* 1996) and it might contribute to a rapid relapse of drug abuse on re-exposition to a drug.

We could not find evidence about the effects of benzodiazepines on morphine sensitization in the literature. The first (and major) aim of the present study was to investigate whether a benzodiazepine alprazolam can influence sensitization to the locomotor activating effects of morphine in mice.

Benzodiazepines can antagonize some effects of opioids. The attenuation of morphine withdrawal syndrome by acute benzodiazepine administration has been well documented (Valverde *et al.* 1995, Gray 1996, Suzuki *et al.* 1996). Benzodiazepines decrease dopamine turnover and release in the nucleus accumbens (Fuxe *et al.* 1975, Di Chiara *et al.* 1991, Finlay *et al.* 1992). In contrast, morphine binding to the opioid receptor in the ventral tegmental area directly inhibits GABAergic neurons, resulting in an increase in mesolimbic dopamine neurotransmission (Johnson and North 1992). Increased release of dopamine causes enhanced locomotor activity (Joyce and Iversen 1979, Vezina *et al.* 1987, Spanagel *et al.* 1993). The reinforcing effect of opioids may also be mediated by the mesolimbic dopamine system (Wise 1989).

The second aim of the present study was to ascertain whether alprazolam can attenuate the development or expression of morphine sensitization. This hypothesis was developed as a result of evidence that benzodiazepines may antagonize some effects of morphine.

A selective serotonin reuptake inhibitor (SSRI) fluoxetine was reported to block the sensitized locomotor stimulating effect to the morphine challenge in rats (Sills and Fletcher 1997). The third aim of our study was to verify this effect in mice and, in case of positive results, to use fluoxetine as a positive control.

The present experiments were designed as an introductory investigation of the effect of alprazolam and fluoxetine on the development and expression of behavioral sensitization in mice. In studies on the development of sensitization, alprazolam or fluoxetine were co-administered with morphine during the period when morphine was administered daily by i.p. injections. The response to morphine was then tested during the sensitization period when the drugs were co-administered

and also 10 days later in the absence of alprazolam or fluoxetine. To study the effect of alprazolam or fluoxetine on the expression of a previously developed sensitized response to morphine, mice first received a daily dose of morphine only; after sensitization had developed, the effect of acute alprazolam or fluoxetine administration on the response to morphine was examined.

Methods

Subjects

Experiments were carried out on adult (6 weeks old) male ICR mice (18-22 g, Velaz, Prague, n=99). Animals were kept under standard laboratory conditions with free access to food and water. Animals were housed ten per cage in a light-controlled room (12-h light/dark cycle, lights on at 7:00 h) and at temperatures ranging from 22 to 24 °C. The measurements were performed under room lighting from 08:00 to 13:00 h.

Experiments were approved by the Expert Committee for Protection of Experimental Animals of the Third Faculty of Medicine and were performed in accordance with the Animal Protection Act of the Czech Republic (No. 246/1992 Sb).

Apparatus

The horizontal locomotor activity of the mice was registered by the Locomotor Activity Apparatus Ugo Basile 7431, containing 16 photocells 3 cm above the floor under transparent cover. Interruptions of light beams to the photocells were recorded during horizontal movement of the animals.

Drugs

Morphine HCl (Sigma-Aldrich, Prague) and fluoxetine HCl (Léčiva, Prague) were dissolved in saline and administered intraperitoneally in a volume 0.1 ml/10 g of body weight. Alprazolam (Léčiva, Prague) was dissolved in distilled water with two drops of Tween 80 and administered orally in a volume 0.2 ml/10 g of body weight.

Procedure

Sensitization was produced by daily injections of 10 mg/kg of morphine i.p. for 10 days (sensitization period). The response to morphine was measured on the first, 5th and 10th day of the morphine treatment (the first, second and third measurement, respectively). Ten days after administration of the last sensitization dose of morphine, the effects of the challenge dose of morphine

(10 mg/kg i.p.) were also measured (i.e. the fourth measurement) (Covington and Miczek 1999).

In studies on the development of sensitization, alprazolam (0.25 mg/kg p.o.) or fluoxetine (5 mg/kg i.p.) were co-administered daily with morphine during the whole 10-day sensitization period. The response to morphine was tested on day 1, 5 and 10 of the sensitization period (when alprazolam or fluoxetine were co-administered) and also 10 days later after the administration of the last sensitization dose of morphine in the absence of alprazolam or fluoxetine (so as to dissociate any effects of other substances in morphine-induced sensitization).

To study the effect of alprazolam or fluoxetine on the expression of a previously developed sensitized response to morphine, mice first received a daily dose of morphine (10 mg/kg i.p.) for 10 days. Ten days after the administration of the last sensitization dose of morphine, the effect of acute alprazolam (0.25 mg/kg p.o.) or fluoxetine (5 mg/kg i.p.) administration on the response to the challenge dose of morphine (10 mg/kg i.p.) was examined.

Alprazolam was administered 30 min prior to the morphine dose, fluoxetine was given together with morphine. A corresponding vehicle was given as the control treatment. Mice were randomly allocated to

corresponding treatment groups (n=12-13/group). Immediately after each administration of morphine, mice were placed singly in transparent plastic cages (20×30×20 cm) with wood shavings on the floor and covered with a transparent top with apertures for air for one hour. Locomotor activity was measured for 3 min one hour after the morphine administration on appropriate days in the same cage.

Dosage for alprazolam, morphine and fluoxetine were derived from our previous studies (Kršiak and Šulcová 1990) and literature (Hascoet and Bourin 1997, Kuribara 1996, Sills and Fletcher 1997) and checked in a pilot experiment. Selected doses of alprazolam and fluoxetine did not influence locomotor activity in the present activity cage.

Statistical analysis

The locomotion data from both parts of the experiment were collected and statistically evaluated by two-way analysis of variance (ANOVA) for repeated measurements with treatment as one factor and measurements as the second factor. When appropriate, comparisons between treatment groups or measurements were conducted using Tukey *post-hoc* test, $P < 0.05$ was considered significant.

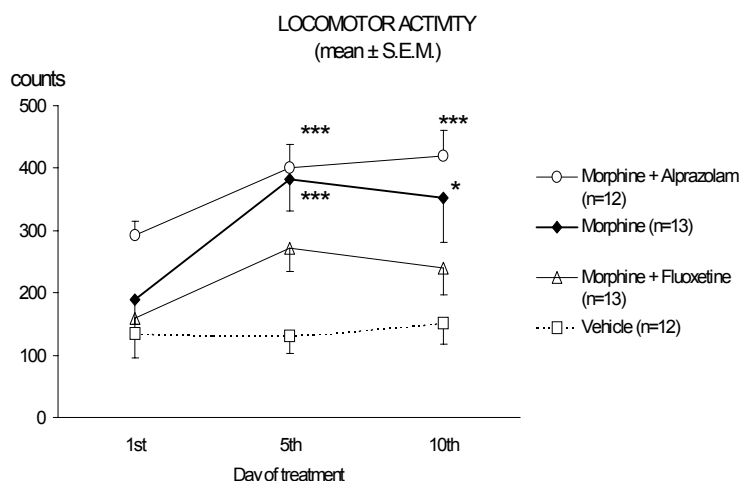


Fig. 1. Effect of alprazolam (0.25 mg/kg) or fluoxetine (5 mg/kg) co-administration with morphine (10 mg/kg) on the development of sensitization to locomotor stimulant effect of morphine in mice. Drugs (or vehicle) were given daily for 10 days. Locomotor activity was measured in photocell activity cages (for 3 min one hour after the morphine administration). *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared to the vehicle-treated (control) group of mice (a two-way repeated measures ANOVA with a *post-hoc* Tukey test).

Results

Two-way repeated ANOVA analysis showed a significant effect of treatment ($F(7,273)=5,548$, $p < 0.001$), measurement ($F(3,273)=21.335$, $p < 0.001$) and interaction treatment \times measurement ($F(21,273)=2,649$, $p < 0.001$) in the number of locomotor activity counts. No significant differences between treated groups of mice were seen in the first measurement (first day of treatment).

Development of sensitization to morphine

Morphine produced more marked increases in locomotor activity after repeated administration than the vehicle only. The difference was significant on day 5 and 10 of treatment ($q=5.399$, $p=0.001$ and $q=4.320$, $p=0.019$, respectively, Tukey test), as compared with the group of mice treated with the vehicle only (Fig. 1). Co-administration of alprazolam with morphine did not

influence the development of sensitization: locomotor activity of this group of mice progressively increased with repeated administrations, similarly to the morphine group, with significant increases on the 5th and 10th day of treatment ($q=5.674$, $p<0.001$ and $q=5.646$, $p<0.001$, respectively, Tukey test) in comparison with the group of mice treated with the vehicle only (Fig. 1). On the other

hand, co-administration of fluoxetine with morphine seems to attenuate the development of sensitization to morphine. Locomotor activity of the morphine + fluoxetine group was lower than that of the morphine group and it did not significantly differ from that of the vehicle group. However, it was still higher than the activity of the control vehicle group (Fig. 1).

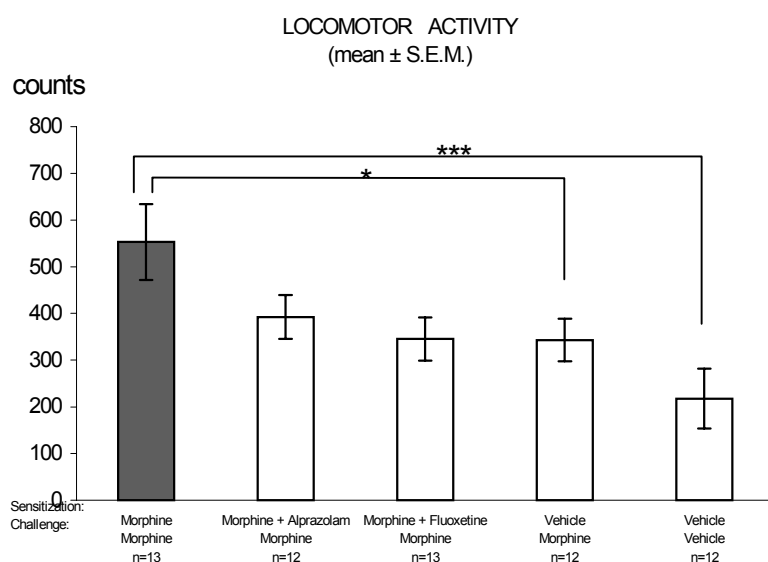


Fig. 2. Effect of challenge dose of morphine (10 mg/kg) in mice treated during sensitization period with morphine (10 mg/kg), morphine (10 mg/kg) and alprazolam (0.25 mg/kg), morphine (10 mg/kg) and fluoxetine (5 mg/kg) or vehicle only. The challenge dose of morphine was given 10 days after termination of sensitization. Drugs (or vehicle) were given daily for 10 days during the sensitization period. Locomotor activity was measured in photocell activity cages (for 3 min one hour after the morphine administration). * $p<0.05$, *** $p<0.001$ for the difference between groups of mice (a two-way repeated measures ANOVA with post-hoc Tukey test).

Qualitatively similar results were obtained with the challenge dose of morphine tested in the absence of alprazolam or fluoxetine 10 days after termination of the sensitization treatment (Fig. 2). Mice sensitized with morphine showed the largest increase of locomotion in comparison with the mice given the vehicle only during the sensitization period and on receiving the challenge treatment ($q=7.187$, $p<0.001$, Tukey test). This increase was also significant in comparison with the mice given vehicle during the sensitization period but challenged with morphine ($q=4.504$, $p=0.013$, Tukey test). Mice sensitized with morphine in co-administration with alprazolam exhibited lower increase of locomotion after the challenge dose of morphine than mice sensitized solely with morphine, but this difference was not significant (Fig. 2). However, mice sensitized with morphine in co-administration with fluoxetine showed an even smaller increase of locomotion after the challenge dose of morphine. Inspection of Figure 2 suggests that the response to the challenge dose of morphine of mice

sensitized to morphine in co-administration with fluoxetine actually did not differ from that of morphine-naive mice (given vehicle only during the sensitization period).

Expression of sensitization to morphine

As shown in Figure 3, mice with a previously developed sensitized response to morphine responded to the challenge treatment with morphine significantly more strongly than to the challenge with the vehicle ($q=5.775$, $p<0.001$, Tukey test). Acute pretreatment with alprazolam did not influence the effect of the challenge dose of morphine. Locomotion of this group of mice was significantly higher than that of the morphine-sensitized mice challenged with the vehicle ($q=5.392$, $p=0.003$). On the other hand, fluoxetine administered with the challenge dose of morphine reduced its locomotor stimulating effect and this group of mice did not significantly differ from other groups of mice (Fig. 3).

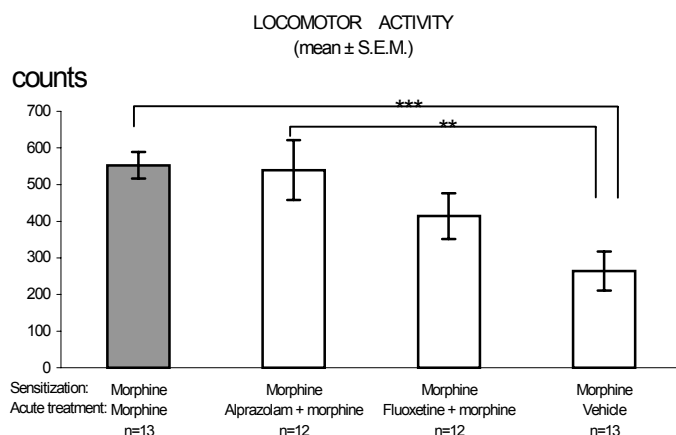


Fig. 3. Effect of acute treatment with alprazolam (0.25 mg/kg) or fluoxetine (5 mg/kg) on the expression of sensitization to locomotor stimulant effect of morphine (assessed according to effects of a challenge dose 10 mg/kg of morphine) in mice. Locomotor activity was measured in photocell activity cages (for 3 min one hour after the morphine administration). ** $p < 0.01$, *** $p < 0.001$ for the difference between groups of mice (a two-way repeated measures ANOVA with post-hoc Tukey test).

Discussion

Mice given morphine repeatedly according to the present experimental protocol (injections of 10 mg/kg of morphine daily for 10 days) exhibited a much greater increase in locomotor activity after morphine than those receiving chronic treatment with the vehicle. This difference in response to morphine between the groups indicates the presence of sensitization.

Morphine produced a more marked increase in locomotor activity than the vehicle during the sensitization period, regardless of whether or not alprazolam was co-administered. However, the effect of the challenge dose of morphine alone was somewhat smaller in mice sensitized with morphine plus alprazolam than in the mice sensitized with morphine only. This suggests that the alprazolam dependence might participate in morphine sensitization. Indeed, mice sensitized to amphetamine with chlordiazepoxide failed to show a sensitized response (augmented locomotion) when challenged with amphetamine alone (Stephens *et al.* 2000).

On the other hand, alprazolam administered acutely before the challenge dose of morphine did not affect the sensitization in mice previously sensitized with morphine alone. In this case, the sensitized response was not influenced by alprazolam in spite of a change in the challenge condition.

Thus, we could not find any significant effect of alprazolam on the development or expression of locomotor sensitization to morphine in these experiments. This negative result does not seem to be due to the ineffectiveness of the selected dose of alprazolam. Although the dose 0.25 mg/kg of alprazolam *per se* did not influence the locomotor activity of mice in the present study, it was not behaviorally ineffective in mice in

another study from our laboratory. A much lower dose of alprazolam (0.05 mg/kg) given by the same route and at the same pre-test interval produced a number of significant behavioral changes in a social conflict in mice (Kršiak and Šulcová 1990).

The sensitized locomotor response to the challenge dose of morphine was partly reduced by co-administering fluoxetine with morphine during the sensitization period (modeling a sort of preventive therapy). The present experiments thus showed similar results in mice to the results reported earlier in rats (Sills and Fletcher 1997). Moreover, the acute administration of fluoxetine with the challenge dose of morphine (modelling acute therapy) also partly reduced a sensitized response to morphine in the present study. Although a change in drug administration could also play a role in the effect of the morphine challenge in the fluoxetine part of this study, the results could hardly depend on the inhibitory effect of fluoxetine on the development of morphine sensitization during the sensitization period (on day 5 and 10 of treatment). These results also indicate that the present experimental arrangement could detect experimental therapeutic effects of drugs on the behavioral sensitization to morphine.

In conclusion, the present experiments have not yielded evidence to support the idea that a benzodiazepine, alprazolam, may influence behavioral sensitization to morphine. However, they corroborated and extended the results indicating that fluoxetine can attenuate to a certain extent the development and expression of morphine sensitization.

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