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RESEARCH ARTICLE

Effect of oral yohimbine on withdrawal jumping behaviour of morphine-dependent mice

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Abstract

Acute administration of the alpha-2 adrenoceptor agonist clonidine and chronic administration of the alpha-2 antagonist yohimbine both inhibit opioid withdrawal signs in experimental models of dependence and also in clinical studies with opiate abusers. There are exceptions to this general rule: restlessness or self-reported abstinence in humans and withdrawal-induced escape behaviour in rodents are resistant to inhibition by acute clonidine. We have explored the effect of the alpha-2 antagonist yohimbine on morphine withdrawal-induced escape behaviour in a mouse model that we have proposed to differentiate between the urge to escape (number of jumps) and non-specific sedative/motor actions (height of jumps). Morphine dependence was induced by s.c. administration of a sustained-release preparation (1 g/kg). Naloxone (1 mg/kg) was injected to precipitate withdrawal jumping 72 hours after morphine injection. Co-treatment with yohimbine dissolved in the tap water (70 mg/l) decreased the number of jumps upon naloxone challenge, an effect which did not seem to be related with a sedative or toxic effect of the drug. This result confirms previous data and suggests that yohimbine could prevent the development of opioid dependence being active to decrease withdrawal-induced escape behaviour. The mechanisms of this action are discussed.

Introduction

The alpha-2 adrenoceptor agonist clonidine is used widely to relieve the symptoms of opioid withdrawal in humans; the drug is markedly effective on autonomic hyperactivity, but some other variables such as restlessness or the subject-rated withdrawal experience seem to be less affected.[1,2] Studies performed with laboratory animals provided similar results: while clonidine blocks many of the signs of opioid withdrawal in the rat, there is an increase in the urge to escape from the enclosure where the animals are confined during the behavioural scoring of abstinence.[3] Morphine withdrawal jumping in mice, which can be considered a kind of escape behaviour, is also potentiated by clonidine in some studies.[4]

Jumping behaviour can be evaluated by either the number, height or distribution of jumps.[5] We have developed an automatic device to study the different components of jumping behaviour and have shown that clonidine tends to potentiate

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naloxone-induced jumping in morphine-dependent mice, but it lowers the height of the jumps performed.[6] On the basis of these experiments, we have hypothesized that the number of jumps could be a reliable index of "urge to escape" reflecting dysphoria, while a decreased height of the jumps could reflect sedative or muscle relaxant effects. According to this idea, in the case of clonidine the potentiation or lack of effects on the total number of jumps performed by opioid-abstinent mice could predict a lack of positive effects on subject-rated withdrawal in abstinent humans; on the other hand, the decrease of the height of jumps provided by this drug could be indicative of sedation. It appears, therefore, that some signs of behavioural abstinence could be resistant to alpha-2 agonists such as clonidine.

Concerning the actions of alpha-2 adrenergic antagonists on opioid abstinence, it has been shown that these agents increase withdrawal scores when given acutely to opioid-abstinent laboratory animals,[7] a finding consistent with the antiwithdrawal actions of alpha-2 agonists; however, the alpha-2 blocker yohimbine is able to prevent the development of morphine tolerance *in vitro*[8] and morphine dependence *in vivo*[9-11] if it is co-administered with the opioid. Preliminary clinical data also show that yohimbine administration for 7 days decrease the intensity of precipitated withdrawal in methadone addicts.[12] These results converge with others, showing that opioid tolerance and dependence involve adaptational changes of alpha-2 adrenergic function.[13,14] It must be pointed out that escape attempts in rats[9,10] and jumping in mice[11] are among the opioid withdrawal signs effectively prevented by yohimbine, in spite of the fact that these variables were resistant to acute doses of clonidine; nevertheless, the methods used in previous studies were not able to differentiate between the urge to escape and a non-specific decrease of the intensity of the escape attempts. This work tries to determine if yohimbine effectively antagonizes the escape response to opioid withdrawal by studying this behaviour in a mouse experiment which permits determination of the number, distribution and height of jumps.[6]

Materials and methods

Male OF1 mice (IFFA-CREDO, France) weighing 30–35 g were used. Animals had free access to tap water and standard diet (UAR A04, France) and were maintained in a controlled environment (20–24°C temperature, 12-hour/12-hour dark/light cycle). Morphine dependence was induced by s.c. administration (10 ml/kg) of a sustained-release preparation of morphine base (1 g/10 ml) containing 50% NaCl, 42.5% paraffin oil and 7.5% arlacel A.[6] From 1 hour to 70 hours after morphine treatment, half the animals were group-housed and had free access to drinking bottles containing a solution of yohimbine in tap water (70 mg/l) exclusively, while the others drank normal tap water. The solution of yohimbine was changed every 24 hours and the amount of fluid consumed was determined at these intervals. Seventy-two hours after morphine, animals received naloxone (1 mg/kg, i.p.) and were placed in a jump meter to record jumping behaviour for 10 min.

Jumping behaviour of morphine-abstinent mice was recorded as described previously by means of a CIBERTEC CA-85 jump meter.[6] Briefly, this apparatus consisted of four individual Plexigas cylinders with three sets of photocell detectors placed around each of them at three different heights (level 1, 12 cm; level 2, 17 cm; level 3, 22 cm). The recorder shows the number of jumps executed by the animals classified by their potency, which was estimated by the highest detector level reached.

Statistical analysis was performed with ANOVA followed by multiple range tests (least significant differences) for multiple comparisons. A probability of 0.05 or less was considered significant.

Results

The mean doses of yohimbine consumed by the animals were 7.0 mg/kg from 0 to 24 hours, 11.2 mg/kg from 24 to 48 hours and 12.3 mg/kg from 48 to 70 hours. Since animals were group-housed, a measure of variability for yohimbine intake cannot be provided.

Oral yohimbine was able to decrease naloxone-induced jumping with respect to the group of morphine-treated animals that drank from a tap-water bottle, as indicated by a significant reduction of the total number of jumps and the jumps of intermediate height (level 2, Fig. 1). Animals in the yohimbine group also exhibited a somewhat different jumping activity concerning the distribution of jumps, with a faster decay along the 10-min observation period (Fig. 2). The mortality

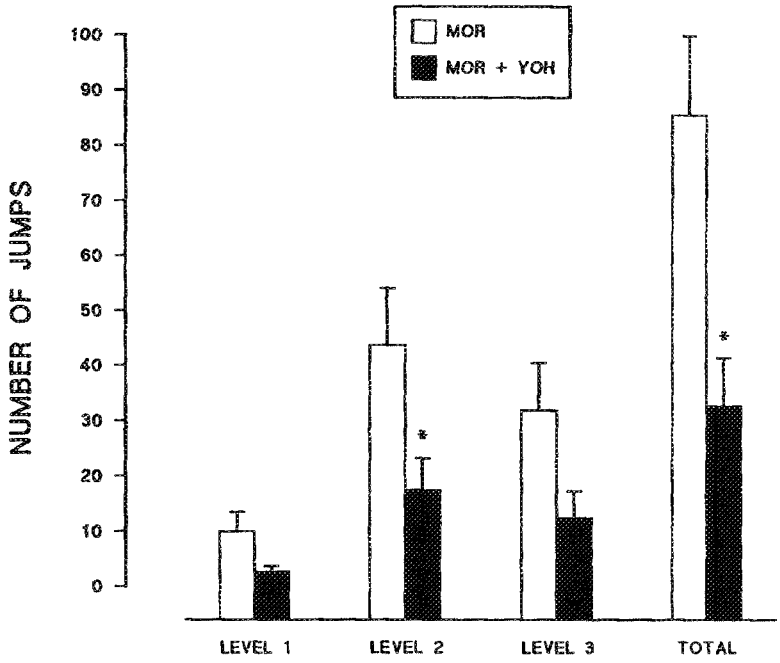

Figure 1. *Jumping activity elicited by naloxone administration to mice rendered opioid-dependent by administration of a sustained-release morphine suspension (1 g/kg, s.c.). Morphine-treated animals had free access to a yohimbine solution as drinking supply (MOR + YOH group, n = 11) or normal tap water (MOR group, n = 12). Jumps are classified by the highest level reached, ranging from 1 (slight jumps) to 3 (potent jumps). Bars represent mean and SEM values.* $^{*}p < 0.05$ *vs MOR group.*

observed in the yohimbine group (1/12) was not statistically different from that obtained in the group drinking normal tap water (4/16).

Discussion

The results show that oral yohimbine antagonizes the development of morphine dependence in mice and therefore the expression of withdrawal-induced escape behaviour. The pharmacokinetics of yohimbine has not been deeply studied, the only data available showing an incomplete oral bioavailability in man,[15] but a rapid entry into the brain of both mice and rats.[16,17] In our mouse experiment, the decrease of morphine dependence was obtained with mean oral doses which could be expected to provide significant brain levels and which are close to those used in clinical studies; however, the scarce knowledge of yohimbine pharmacokinetics in the mouse and the lack of data on the variability of yohimbine intake preclude any quantitative assumption from our results. The experiments performed did not indicate a non-specific effect of yohimbine on the mice ability to jump, since this drug neither increased the apparent toxicity of the treatment nor affected specifically the most potent jumps; on the contrary, it was more effective on jumps of lower intensity. The distribution of jumps was slightly affected by yohimbine, but this change is difficult to interpret.

These results together with previous evidence[9-11] are in agreement with the idea that prevention of morphine dependence by yohimbine not only involves the autonomic response of withdrawal, but also behavioural signs probably more related to psychological abstinence. Although the first clinical study performed with yohimbine on methadone addicts is very limited, it showed that yohimbine 10 mg/kg given three times a day for 1 week produced a 30% decrease of naloxone-precipitated withdrawal severity, assessed by the subjects with the aid of a visual analogue scale.[12] It appears, therefore, that yohimbine could be of interest in the therapeutic management of opioid dependence.

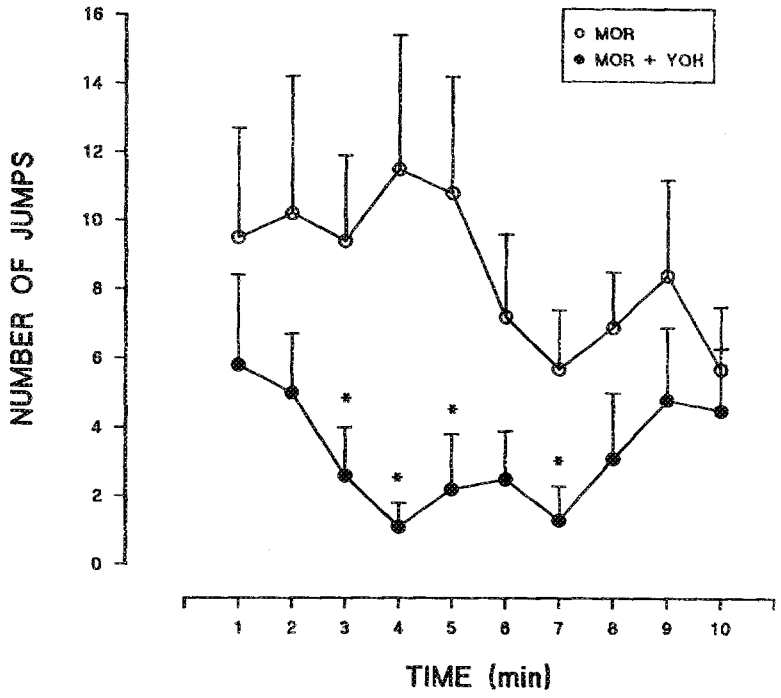

Figure 2. *Distribution of the jumps elicited by naloxone administration to mice rendered opioid-dependent by administration of a sustained-release morphine suspension (1 g/kg, s.c.). Morphine-treated animals had free access to a yohimbine solution as drinking supply (MOR + YOH group, n = 11) or normal tap water (MOR group, n = 12). Points represent the cumulative number of jumps (mean ± SEM) for each minute along a 10-min observation period.* $^{*}p < 0.05$ *vs MOR group.*

The mechanism involved in the effect of yohimbine on opioid tolerance and dependence has not been studied extensively. The only experiments performed so far ruled out a possible yohimbine-induced reversal of the beta-adrenoceptor upregulation seen in cerebral cortex of morphine-dependent rats.[18] Taking into account that yohimbine is an effective alpha-2 adrenoceptor antagonist, it could be argued that the anti-withdrawal effect of this drug could be related to a specific blockade of alpha-2 adrenoceptors during morphine exposure, similar to the effect described *in vitro* on morphine tolerance.[8] Although the effects of acute clonidine on withdrawal jumping are not in good agreement with this idea, recent evidence show that clonidine could have mixed actions in this situation. Thus, Sharif & El-Kadi[4] have shown that acute clonidine potentiate jumping probably by acting on imidazoline receptors at high doses, while low doses of this drug seem to decrease jumping in a yohimbine-sensitive manner.

The possible participation of other pharmacological mechanisms in the effect of yohimbine must be discussed, however. It must be pointed out that yohimbine also modifies serotonergic pathways, which in turn are involved in opioid withdrawal jumping;[19] thus, yohimbine increases extracellular 5-hydroxytriptamine (5-HT) levels in the rat frontal cortex[20] and those of the metabolite 5-hydroxyindolacetic acid in the rat lateral ventricular fluid.[21] These serotonergic actions could be partially mediated by an alpha-2 adrenoceptor blockade, since other drugs also enhance 5-HT transmission in the rat by blocking alpha-2 adrenoceptors in areas such as the hippocampus.[22] However, some of the actions of yohimbine could be directly mediated by 5-HT receptors: binding studies have revealed a high affinity of yohimbine for rat brain 5-HT_{1A} receptors ($K_D = 74$ nM) and, interestingly, rats trained with yohimbine as a discriminative stimulus generalize to drugs with minimal affinity for the alpha-2 adrenoceptor but with high affinity for 5-HT_{1A} receptors.[23] The possible contribution of 5-HT mechanisms to the action of yohimbine on mor-

phine dependence is thus reasonable and must be clarified; testing the anti-withdrawal properties of drugs more specific for the different receptors operated by yohimbine could add precise information on the mechanisms involved in opioid dependence prevention.

In summary, the present experiments confirm that yohimbine prevents the development of morphine dependence in rodents; moreover, this effect does not affect autonomic withdrawal symptoms exclusively, but also complex behaviours such as attempts to escape from a closed environment. The mechanism of this action remains currently unknown.

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