A pharmacological modulation of opiate withdrawal using an up-/down-regulation of the noradrenergic system in opiate-dependent rats

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Abstract

Chronic opioid exposure induces neuroadaptative changes in several brain systems. Amongst others the alpha adrenergic system appears to be extremely sensitive to opioid exposure and has, therefore, been proposed to play a key role in opiate withdrawal symptoms. In order to better understand the influence of the noradrenergic system in opioid withdrawal and be able to develop new therapeutic strategies, we studied the effect of pre-treatment with the \( \alpha_2 \) agonist (clonidine) and \( \alpha_2 \) antagonist (yohimbine) on naloxone-precipitated withdrawal in opiate-dependent rats. As is already known clonidine pre-treatment significantly enhances autonomic and behavioural signs of opioid withdrawal whereas yohimbine significantly attenuates them with dose-related effect. We also tested the effect of clonidine (0.1 mg/kg) during naloxone-precipitated opiate withdrawal in rats pre-treated with yohimbine (5 mg/kg) and we observed that yohimbine pre-treatment potentiates clonidine efficiency in decreasing opiate withdrawal signs. This study supports the possibility of using a noradrenergic antagonist in order to regulate adrenoceptors chronically exposed to opioids, therefore interfering with the intensity of naloxone-precipitated opiate withdrawal and potentiating later effectiveness of noradrenergic agonists like clonidine. These results may have various applications in clinical opiate detoxification protocols and are discussed through an up-/down- regulation of adrenoreceptors.

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Introduction

In the last few years, there has been an increasing interest in the development of new therapeutic strategies in the management of opiate detoxification. Most approaches have concentrated on diminishing withdrawal symptoms, which have been shown to be mostly related to noradrenergic activity (Funada et al., 1994; Redmond and Huang, 1982). Chronic opiate administration induces some neuroadaptative changes, including up-regulation of the cAMP system (Nestler and Tallman, 1988; Nestler et al., 1989; Nestler, 1992). These adaptations are thought to compensate for opiate-mediated neuronal disturbances as well as contributing to opiate dependence. A model system for these neuroadaptative changes is the locus coeruleus (LC), which is the major noradrenergic nucleus in the brain (Maldonado et al., 1996). Chronic opiate exposure is associated with increased expression of adenylyl cyclase and cAMP-dependent protein kinase in the LC (Maldonado et al., 1996).
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This up-regulation of the cAMP pathway probably contributes to the electrical hyperexcitability of LC neurons during opiate withdrawal (Laverty and Roth, 1980). Furthermore, it has been demonstrated that administration of the $\alpha_2$ agonist clonidine results in an inhibitory effect on LC activity, related to common transduction mechanisms between opioids and $\alpha_2$ receptors (Aghajanian, 1978). Therefore, it could be expected that $\alpha_2$ agonists mimic morphine action in the LC and up-regulate the cAMP system while $\alpha_2$ antagonist down-regulate the same system (El Kadi and Sharif, 1997). Clonidine is now widely used in clinical practice to reduce acute opiate withdrawal symptoms. Clonidine is shown to effectively reduce acute opiate withdrawal in animals and humans (Gold et al., 1978; Katz, 1986). Furthermore, as shown by several authors (e.g. Kalso et al., 1993; Solomon and Gebhart, 1988) chronic opioid administration leads to modifications in the sensitivity to noradrenergic drugs like clonidine. Because of cross-tolerance between opioids and noradrenergic receptors, clinicians must, therefore, use larger doses of clonidine in the management of acute opioid withdrawal in patients.

While $\alpha_2$ adrenergic agonists benefit from a large interest in various opiate detoxification protocols including ‘rapid detoxification’ under anaesthesia or sedation (Streel and Verbanck, 2003), the $\alpha_2$ adrenergic antagonists appear to be underestimated. Moreover, regarding potential noradrenergic regulation, promising results of $\alpha_2$ adrenergic antagonist yohimbine pre-treatment were found in mice (El Kadi and Sharif, 1997) and rats (Taylor et al., 1991). To our knowledge only one team has reported the use of this approach in humans (Hameedi et al., 1997). Pre-clinical data documenting the effect of yohimbine pre-treatment on opiate withdrawal expression are still needed to evaluate the potential therapeutic application of yohimbine in opiate detoxification. Nevertheless the combined use of $\alpha_2$ adrenergic antagonists and agonists should be as promising as the use of $\alpha_2$ adrenergic agonists alone in the potential interference of opiate withdrawal.

Thus, considering that opioid treatment leads to an up-regulation of adrenoreceptors, we hypothesize that pre-treatment with the noradrenergic antagonist yohimbine should down-regulate the adrenoreceptors and decrease the expression of naloxone-precipitated opiate withdrawal in opiate-dependent rats, while pre-treatment with the noradrenergic agonist clonidine should produce the opposite effect. Furthermore, pre-treatment with yohimbine could potentiate the efficiency of clonidine in reducing opiate withdrawal signs. These results may come to have a certain impact and may lead to useful modifications in the management and implementation of new opioid detoxification protocols in humans.

Methods

Male Wistar rats weighing 250–300 g were individually housed in plastic cages with free access to food and water for 1 wk prior to the experiment. Morphine dependence was induced by multiple injection of the drug [subcutaneously (s.c.), in the scruff of the neck] following a modified version of a schedule previously used (Streel et al., 2000, 2001). The protocol has been approved by the Ethical and Animal Welfare Committee of the university (Université Libre de Bruxelles).

Experiment 1

Morphine and saline treatment

During 3 d, the rats received increasing doses of morphine twice a day (at 10:00 and 16:00 hours) as follows (in mg/kg of body wt): day 1 (10, 20), day 2 (30, 40), day 3 (50, 50). During the next 3 d, the rats received a regular dose of morphine once a day (at 12:00 hours). The doses were as follow (in mg/kg of body wt): day 4 (50), day 5 (50) and day 6 (50). Saline-treated rats received saline instead of morphine. Therefore, 40 rats were treated with morphine (Mor) and 40 rats were treated with saline (Sal).

Clonidine (Clo), yohimbine (Yoh) and saline treatment

On day 4, morphine-treated ($n = 40$) and saline-treated ($n = 40$) rats were assigned to height groups. During days 4–6, each group was given clonidine (10 mg/kg) or yohimbine (5 mg/kg or 10 mg/kg) or saline by s.c. injection 15 min after the regular dose of morphine or saline (at 12:15 hours). The groups were as follows:

Morphine-treated rats. Mor–Sal (received saline, $n = 10$); Mor–Clo (received 10 mg/kg clonidine, $n = 10$); Mor–Yoh5 (received 5 mg/kg yohimbine, $n = 10$); Mor–Yoh10 (received 10 mg/kg yohimbine, $n = 10$).

Saline-treated rats. Sal–Sal (received saline, $n = 10$); Sal–Clo (received 10 mg/kg clonidine, $n = 10$); Sal–Yoh5 (received 5 mg/kg yohimbine, $n = 10$); Sal–Yoh10 (received 10 mg/kg yohimbine, $n = 10$).

On day 6, 2 h after the last injections of morphine and various pre-treatments (at 14:15 hours), withdrawal was precipitated using s.c. injection of naloxone (1 mg/kg of body wt). During the 15 min following naloxone injection, the rats were placed in...
transparent cages and observed for withdrawal quantification. Global withdrawal score (GWS) was calculated by attributing one point for each of the following signs: ‘wet-dog shakes’, jumping, head lift, profuse salivation, escape attempts, vocalization when touched, abnormal posture, mastication, teeth chattering, cheek tremors, sniffing.

Experiment 2

Morphine and saline treatment

During 3 d, the rats received increasing doses of morphine twice a day (at 10:00 and 16:00 hours) as follows (in mg/kg of body wt): day 1 (10, 20), day 2 (30, 40), day 3 (50, 50). During the next 3 d, the rats received a regular dose of morphine once a day (at 12:00 hours). The doses were as follow (in mg/kg of body wt): day 4 (50), day 5 (50) and day 6 (50). Saline-treated rats received saline instead of morphine. Therefore, 20 rats were treated with morphine and 20 rats were treated with saline.

Yohimbine and saline treatment

On day 4, morphine-treated (n = 20) and saline-treated (n = 20) rats were assigned to four groups (two morphine and two saline). During days 4–6, each group was given yohimbine (5 mg/kg) or saline by s.c. injection 15 min after the regular dose of morphine or saline (at 12:15 hours). The groups were as follows:

Morphine-treated rats. Mor–Sal (received saline, n = 10); Mor–Yoh (received 5 mg/kg yohimbine, n = 10).

Saline-treated rats. Sal–Sal (received saline, n = 10); Sal–Yoh (received 5 mg/kg yohimbine, n = 10).

On day 6, 2 h after the last injections of morphine or saline and pre-treatments (saline or yohimbine) withdrawal was precipitated using s.c. injection of naloxone (1 mg/kg of body wt). One minute after naloxone injections the rats received clonidine (0.1 mg/kg s.c.). The GWS was calculated and compared.

For both experiments we performed analysis of variance (ANOVA) followed by Bonferroni post-hoc tests in order to avoid errors due to multiple comparisons. The results were considered significant when the probability level was <0.05.

Results

Experiment 1

ANOVA shows significant differences in GWS after naloxone-precipitated opiate withdrawal \([F(7, 56) = 69.78, p \leq 0.0001]\). Post-hoc analyses are as follows (see Table 1).

Experiment 2

ANOVA shows significant differences in the GWS after naloxone-precipitated opiate-withdrawal followed by clonidine (0.1 mg/kg), \([F(3, 20) = 58.64, p \leq 0.0001]\). Post-hoc analyses are as follows: Mor–Yoh vs. Mor–Sal (p < 0.001); Mor–Yoh vs. Sal–Yoh (p < 0.001); Mor–Yoh vs. Sal–Sal (p < 0.001); Mor–Sal vs. Sal–Yoh (p < 0.001); Mor–Sal vs. Sal–Sal (p < 0.001) and Sal–Yoh vs. Sal–Sal (n.s.). It is obvious but important to note that in the absence of an opiate-treatment regimen the rats do not score on the GWS after naloxone administration. The effects observed in opiate-treated rats (see Figures 1 and 2) are, therefore, attributable to the various treatments with yohimbine, clonidine or saline only in the presence of morphine dependence.
Discussion

Experiment 1 was designed in order to show potential effects of pre-treatments with clonidine or yohimbine on GWS. Experiment 2 was designed, according to the idea of a potential noradrenergic modulation, to test if it was possible to potentiate clonidine effect on opiate withdrawal signs through yohimbine pre-treatment. As Experiment 2 was also designed to better approach a clinical model, we greatly reduced the doses of clonidine.

Our study shows that morphine-dependent rats pre-treated with yohimbine (10 mg/kg of body wt) consistently present a significant decrease in GWS expression in comparison with a control group pre-treated with saline.

The GWS expression in the group pre-treated with clonidine was more than five times higher than the group pre-treated with 10 mg/kg yohimbine. Furthermore, as showed by Experiment 2, clonidine (0.1 mg/kg s.c.) is more efficient in reducing opiate withdrawal signs after yohimbine (10 mg/kg) pre-treatment in comparison with saline treatment. As previously by Taylor et al. (1991) yohimbine could produce anti-withdrawal effects by an increased neuronal activity that could oppose morphine inhibition of LC noradrenergic neurons. However, this model has been questioned by some authors (e.g. Ambrosio et al., 1997) considering that others (e.g. Illes and Norenberg, 1990) have shown that blockade of $\alpha_2$ adrenoreceptors increases opioid receptor inhibition of the firing rate of rat LC (Illes and Norenberg, 1990) and that, accordingly, administration of yohimbine should potentiate rather than decrease opiate withdrawal.

Nevertheless, the effect of yohimbine could be understood in an alternative way, considering a system involving an up-/down-regulation of noradrenergic receptors. In this view, pre-treatment with $\alpha_2$ antagonist would down-regulate the cAMP pathway leading to a decrease in the hypoexcitability of LC neurons induced by chronic morphine treatment. A decrease in this hypoexcitability would reduce the

![Figure 1. Effects of clonidine or yohimbine pre-treatment on opiate withdrawal expression. The Mor–Sal group (■) was pre-treated with saline, the Mor–Yoh5 group (□) was pre-treated with 5 mg/kg yohimbine, the Mor–Yoh10 group (■) was pre-treated with 10 mg/kg yohimbine, the Mor–Clo group (□) was pre-treated with 5 mg/kg clonidine. Results are expressed in average number of withdrawal signs.](image1)

![Figure 2. Effect of yohimbine in reduction of opiate withdrawal and potentiation of clonidine effectiveness. The Mor–Sal–Clo group (□) was pre-treated with saline and received clonidine (0.1 mg/kg s.c.) after naloxone-precipitated withdrawal, the Mor–Yoh5–Clo group (■) was pre-treated with 5 mg/kg yohimbine and received clonidine (0.1 mg/kg s.c.) after naloxone-precipitated withdrawal; the Mor–Yoh5 group (□) was pre-treated with 5 mg/kg yohimbine and did not receive clonidine after naloxone-precipitated withdrawal. Results are expressed in means of number of withdrawal signs (GWS score).](image2)
subsequent noradrenergic hyperexcitability induced by abrupt blockade of opiate receptors by naloxone. This would account for the lower intensity of opiate withdrawal signs. Similarly, the aggravating effect of clonidine pre-treatment on withdrawal signs is consistent with an up-regulation of noradrenergic activity. Clonidine pre-treatment would up-regulate the cAMP pathway leading to enhancement of hyperexcitability of the LC neurons. In turn, this would enhance subsequent hyperexcitability induced by abrupt blockade of opiate receptors by naloxone. The absence of effect of pre-treatment with 5 mg/kg yohimbine compared to 10 mg/kg yohimbine possibly reflects dose-dependent effect. These results are in accordance with previous studies (Taylor et al., 1991). Moreover, as showed in Experiment 2, clonidine effects are potentiated in rats pre-treated with yohimbine.

Moreover, cross-tolerance between morphine and clonidine is well documented (e.g. Paul and Tran, 1995) and as a consequence higher doses of clonidine are needed to be efficient in opiate-dependent patients during opiate detoxification procedures. This cross-tolerance seems to occur partly as a consequence of increased efficacy of neuromuscular transmission which is produced by an increase in the probability of transmitter release and an increase in the density of sympathetic innervation (Karunanithi and Lavidis, 2001). Therefore, pre-treatment with yohimbine could potentiate clonidine efficiency via an up-/down-regulation which could through several mechanisms including an interference of neurotransmitter release but also through a re-sensibilization of adrenoreceptors.

A potential up-/down-regulation of adrenoceptors may have a wide field of application in clinical practice, where clinicians are still in urgent need of better opiate detoxification procedures such as management of addiction or post-operative intensive care (McQuay et al., 1999). To our knowledge, only one human study has addressed the question of pre-treatment with yohimbine (Hameedi et al., 1997). This pilot study was limited by sample numbers (12 patients divided into three groups), nevertheless, the results were encouraging. Yohimbine (10 mg in three divided doses) was well tolerated and associated with a decrease of 30% in precipitated withdrawal symptoms (Hameedi et al., 1997). The use of the up-/down-regulation adrenoceptor model does not require an expensive or highly specialized infrastructure and could be used to complete classical opiate detoxification procedures. Our study underlines the potential value of yohimbine pre-treatment in opiate detoxification protocols and the enhancement of opiate withdrawal signs by clonidine if introduced too early. In this study the association of yohimbine pre-treatment/clonidine is more efficient than clonidine alone. However, in rats the LD₅₀ (s.c.) for morphine and clonidine is 109 mg/kg and 108 mg/kg respectively (Lewis, 1996), this might help clinicians to understand the seemingly enormous doses, by human standards, needed in this study. Although medication that increases noradrenaline should be used with care in opiate-dependent patients (Stine et al., 2002), yohimbine appears to be safe in normal subjects and in patients with various diseases (Tam et al., 2001). All reported side-effects, including at doses as high as 350 mg in a diabetic patient, are reversible and resolve spontaneously within a short time after termination of the drug therapy (Tam et al., 2001). Friesen et al. (1993) also suggested a benign course even after massive overdose. Further studies should explore whether the combination of the α₄ antagonist pre-treatment with α₂ agonist administration at withdrawal may be useful in further attenuating opiate withdrawal symptoms in humans. Further research is needed to determine clinical applications of up-/down-regulation of adrenoreceptors in humans.

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Statement of Interest

None.

References


