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Original Article

Effect of Tizanidine, Rilmenidine, and Yohimbine on Naloxon-Induced Morphine Withdrawal Syndrome in Mice

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Abstract

In this study using clonidine (a mixed α_2/I_1 receptors agonist), tizanidine (pure α_2-receptor agonist), rilmenidine (I_1 receptor agonist) and yohimbine (α_2-receptor antagonist), we tried to clarify the role of imidazoline and α_2-receptors in morphine withdrawal syndrome.

Morphine-dependence was induced by administration of increasing doses of morphine in mice. After the last administration of morphine, clonidine (0.3 mg/kg, i.p.), tizanidine (1 and 2 mg/kg, i.p.) and rilmenidine (1.5 and 3 mg/kg, i.p.), with / without pretreatment with yohimbine (1 mg/kg, i.p.) were administered 30 min before naloxone (5 mg/kg, i.p.) challenge. Withdrawal symptoms including: jumping, ptosis, piloerection, tremor and diarrhea were recorded.

Rilmenidine (3 mg/kg) decreased naloxone-induced jumping and this effect was partially inhibited by yohimbine. Rilmenidine (1.5 mg/kg), tizanidine and clonidine had no significant effect on jumping. None of drugs influenced ptosis. All drugs increased piloerection and decreased diarrhea. Clonidine and tizanidine decreased tremor.

We conclude that Imidazoline receptors as well as α_2 receptors are involved in morphine withdrawal symptoms and yohimbine as an α_2-antagonist can suppress at least some effects of imidazoline agonists. It is suggested that α_2-receptors are located down-stream to imidazoline receptors and their blockade can inhibit imidazoline effects.

Keywords: Morphine withdrawal; Tizanidine; Rilmenidine; Yohimbine; Clonidine Imidazoline receptors.

Introduction

Physical dependence to opioids has been described as expression of withdrawal symptoms as a result of abrupt withdrawal of opioids, reduction in opioid doses or administration of opioid antagonists. Many investigations have been done on systems or drugs, which alleviate morphine withdrawal syndrome. These include drugs that influence dopaminergic (1), adrenergic (2), excitatory amino acid (3), purinergic (4), NMDA (5), nitric oxide (6), serotoninergic (7) systems, and also herbal medicines (8, 9). Increased noradrenergic activity has been established as a major biochemical component of withdrawal syndrome during opioid abstinence (10). The activity of noradrenergic neurons in the locus coeruleus is inhibited by opioids and the abrupt withdrawal of opioids causes hyperactivity of the neurons in opioids dependent animals (11). Imidazoline receptors as well as presynaptic

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α_2-adrenoceptors can inhibit the release of noradrenalin and several other neurotransmitters (12). Some investigations have been shown that stimulation of imidazoline receptors can cause a decrease in noradrenalin release and neurons activity in Rostral Ventrolateral Medulla (RVLM) and efferent sympathetic tone (13-15).

The aim of this study was to investigate the role of imidazoline I_1 receptor subtypes on morphine withdrawal symptoms in mice by comparison of clonidine (α_2/I_1 receptors agonist), tizanidine (α_2-receptor agonist) and rilmenidine (I_1 receptor agonist) effects, with/without pretreatment with yohimbine (α_2-receptor antagonist) on control of naloxone-induced morphine withdrawal syndrome.

Experimental

Animals

Male albino mice weighing 20-30 g were obtained from Pasteur institute (Tehran-Iran) and maintained in animal house of Isfahan University of Medical Sciences in 12/12 h light/dark cycle at 21±2°C. They had free access to food and water *ad libitum*.

Drugs

The following drugs were used: morphine sulphate (Tolidaru, Iran), naloxone HCl (Tolidaru, Iran), clonidine HCl, yohimbine HCl, rilmenidine hemifumarate (Sigma, St. Louis, USA) and tizandinie HCl (Novartis, Switzerland). Drugs were dissolved in saline and were injected intraperitoneally (i.p.) in a volume of 10 ml/kg; except morphine, which was administered subcutaneously (s.c.). The control groups received saline.

Induction of morphine dependence

Morphine was injected s.c. to mice at doses of 30 and 45 mg/kg on day 1, 60 and 90 mg/kg on day 2 (8:00 AM and 7:00 PM). On day 3, a single dose of morphine (90 mg/kg) was injected at 8:00 AM (16).

Naloxone precipitated withdrawal syndrome

Withdrawal signs were elicited by i.p. injection of naloxone HCl 2h after the last injection of morphine on day 3. Counted and checked signs were assessed during a 30 min period starting just after naloxone administration. Jumping was counted and checked signs including: ptosis, piloerection, tremor and diarrhea were evaluated over 3×l0 min periods with one point given for presence of each sign during each period (maximum score = 3) (16).

Grouping of animals and administration of drugs

Sixty mice were randomly divided in groups of six in each. All animals were rendered dependent on morphine. After induction of morphine dependence, drugs were administered in animals and withdrawal syndrome was induced by naloxone (5 mg/kg, i.p.) (16).

Experiment 1. Administration of drugs without yohimbine pretreatment

After induction of morphine dependence in animals, group 1 received saline, group 2 clonidine (0.3 mg/kg, i.p.) (17), group 3 tizanidine (1 mg/kg, i.p.), group 4 tizanidine (2 mg/kg, i.p.) (18), group 5 rilmenidine (1.5 mg/kg, i.p.) and group 6 rilmenidine (3 mg/kg, i.p.) (19). Thirty min later, naloxone (5 mg/kg, i.p.) was administered and the signs were checked during a 30 min period.

Experiment 2. Administration of drugs with yohimbine (1 mg/kg, i.p.) pretreatment

Morphine dependence was induced as described previously. Eighty minutes after the last injection of morphine, animals of all groups except group 1 pretreated with yohimbine (1 mg/kg, i.p.) (17, 20). Ten min later, group 1 and 2 received saline, group 3 clonidine (0.3 mg/kg, i.p.), group 4 tizanidine (2 mg/kg, i.p.) and group 5 rilmenidine (3 mg/kg, i.p.). Naloxone (5 mg/kg, i.p.) was administered 30 min later and checked and counted signs were recorded during a 30 min period.

Statistical Analysis

The counted data were expressed as mean± S.E.M.; one-way ANOVA followed by Scheffe test was used to compare data of jumping. Mann-Whitney test was used to compare checked signs data. P values less than 0.05 were considered significant.

Table 1. Effects of clonidine, tizanidine and rilmenidine on checked signs of morphine withdrawal syndrome in mice, without yohimbine pretreatment.

Treatment	Dose (mg/kg, i.p.)	Ptosis	Piloerection	Tremor	Diarrhea
Veh.	-	1 (0–3)	0 (0–3)	2.5 (1–3)	1 (0–3)
Yoh.+Veh.	-	0.5 (0–2)	0 (0–0)	2.5 (2–3)	1 (0–2)
Clo.	0.3	1 (0–1)	2 (1–3)***	0.5 (0–2)**	0 (0–0)**
Yoh.+Clo.	0.3	0 (0–1)	2 (0–2)**	1.5 (1–3)	0 (0–0)**
Tiz.	1	0 (0–2)	2 (0–3)**	0.5 (0–1)**	0 (0–0)**
Tiz.	2	0 (0–1)	2 (1–3)***	1 (0–2)*	0 (0–0)**
Yoh.+Tiz.	2	0 (0–2)	1.5 (1–2)***	2 (1–3)	0 (0–1)
Ril.	1.5	0 (0–1)	1 (0–1)**	1 (1–2)	0 (0–0)**
Ril.	3	0.5 (0–3)	1.5 (1–3)***	1 (1–2)	0 (0–0)**
Yoh.+Ril.	3	0.5 (0–3)	0 (0–2)*	2 (1–2)	0 (0–0)**

The upper numbers show the median, and the numbers in the parenthesis show the range of scores. (n=6). Veh: vehicle; Yoh: yohimbine; Clon: clonidine; Tiz: tizanidine; Ril: rilmenidine. * p <0.05; **p < 0.01; ***p < 0.001; statistically significant between test and control groups, (Mann-Whitney test).

Results and Discussion

Experiment 1. Effects of drugs without yohimbine pretreatment

Clonidine (0.3 mg/kg, i.p.) had no significant effect on naloxone-induced jumping (Figure 1) and ptosis (Table 1), but increased piloerection ($p<0.001$) and decreased tremor and diarrhea significantly ($p<0.01$) (Table 1).

Tizanidine (1 mg/kg, i.p.) didn't produce any significant change in naloxone-induced jumping (Figure 1) and ptosis (Table 1). However, increased Piloerection ($p<0.01$), diminished tremor ($p<0.001$) and diarrhea ($p<0.01$) significantly (Table 1).

Tizanidine (2 mg/kg, i.p.) like clonidine, didn't change naloxone-induced jumping (Figure 1) and ptosis (Table 1) significantly, but increased piloerection ($p<0.001$), diminished tremor ($p<0.05$) and decreased diarrhea ($p< 0.01$) significantly (Table 1).

While rilmenidine at a dose of 1.5 mg/kg had no significant effect on naloxone-precipitated jumping, a two-fold dose (3 mg/kg) significantly ($p<0.05$) reduced number of jumping (Figure 1). Rilmenidine at both doses significantly ($p<0.01$) increased piloerection and decreased diarrhea, but did not change tremor (Table 1).

Experiment 2. Effects of Drugs with yohimbine (1 mg/kg, i.p.) pretreatment

As it is observed in Figure 2, yohimbine (1 mg/kg, i.p.) by itself could not induce any significant change in the number of jumping, also there was not any statistically meaningful difference in number of jumps between animals received yohimbine alone and those of other groups that pretreated with yohimbine and also received either clonidine, tizanidine or rilmenidine.

Pretreatment with yohimbine partially

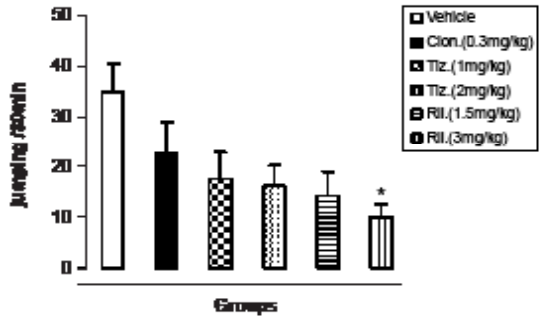

Figure 1. Effects of clonidine (0.3 mg/kg, i.p.), tizanidine (1 and 2 mg/kg, i.p.), and rilmenidine (1.5 and 3 mg/kg, i.p.) on naloxone-induced jumping in morphine-dependent mice. Drugs were administered 30 min prior to naloxone (5 mg/kg, i.p.) injection. Number of jumping was counted during a 30 min period starting just after naloxone injection. The data are expressed as mean S.E.M. (n=6). Clon: clonidine; Tiz: tizanidine; Ril: rilmenidine.
Significant difference between test and control groups is shown as *p < 0.05; (one-way ANOVA followed by Scheffe test).

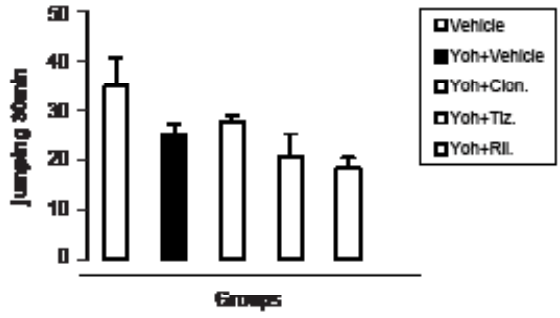

Figure 2. Effects of clonidine (0.3 mg/kg, i.p.), tizanidine (2 mg/kg, i.p.) and rilmenidine (3 mg/kg, i.p.) on naloxone-induced jumpimg in morphine-dependent mice, pretreated with yohimbine (1 mg/kg, i.p.). Drugs were administered 30 min prior to naloxone (5 mg/kg, i.p.) injection. Yohimbine was administered 10 min before drugs. Number of jumping was counted during a 30 min period starting just after naloxone injection. The data are expressed as mean S.E.M. (n=6). Yoh: Yohimbine; Clon: clonidine; Tiz: tizanidine; Ril: rilmenidine. There was no significant difference between groups (p < 0.05) (one-way ANOVA followed by Scheffe).

reversed the effect of rilmenidine on jumping (Figure 2). Yohimbine alone did not change the checked signs of morphine withdrawal, but pretreatment with this drug inhibited reduction of naloxone-induced tremor caused by clonidine or tizanidine (Table 1).

The activity of noradrenergic neurons in the locus coeruleus is inhibited by opioids and the abrupt withdrawal of opioids causes hyperactivity of the locus coeruleus in opioid-dependent animals (11). In the central nervous system (CNS), presynaptic α_2- adrenoceptors inhibit the release of noradrenalin and several other neurotransmitters (12), and it has been shown that stimulation of imidazoline receptors can also cause a decrease in noradrenalin release in Rostral Ventrolateral Medulla (RVLM) (13-15).

In our study, clonidine a mixed α_2/I receptors agonist didn't change naloxone-induced jumping. In agreement with our data, there are other reports showing that clonidine had no effect on jumping (21, 22) also; a report indicates that clonidine potentiated the expression of naloxone induced jumping in morphine-dependent rats (23). Furthermore, there are reports indicating that clonidine given intracerebroventricullarly (i.c.v.) at pico-mol and nano-mol doses, decreased the incidence of morphine withdrawal syndrome in rats and mice, and i.c.v. injection of yohimbine prevented this effect (24). The contrary results from different investigations may be due to different doses of the drug, animal type or a reflection of the effects exerted through α_2-adrenoceptors located on presynaptic or postsynaptic sites of the adrenergic system.

In this study, tizanidine, which is an α_2-adrenoceptor agonist didn't itself alter naloxone-induced jumping in morphine-dependent mice. In contrary to our results, there is a report showing that tizanidine decreased naloxone-induced jumping in morphine-dependent rats (25). This controversy may be due to higher doses of tizanidine were used in their study, which can have muscle relaxant effects and can diminish the strength of muscles for jumping.

Rilmenidine, which is an imidazoline I_1 agonist, decreased naloxone-induced jumping in morphine-dependent mice, which could be partially inhibited by α_2- adrenoceptor antagonist, yohimbine. In agreement with our data, there is a report showing that rilmenidine decreased jumping in morphine-dependent rats (19), and also there are some reports indicate that rilmenidine could inhibit sympathetic tone and decrease blood pressure via effects on imidazoline receptors and these effects were inhibited by administration of α_2- adrenoceptor antagonists (26, 27). They suggested that α_2-adrenoceptors are located down-stream to presynaptic imidazoline receptors that can explain in part the inhibition of imidazoline

effects through α_2- adrenoceptor antagonists; but in our study, although yohimbine could inhibit the effect of rilmenidine on naloxone-induced jumping, this effect was incomplete; therefore other possible mechanisms may be involved. We suggest that the increase of noradrenalin release induced by an α_2-adrenoceptor antagonist can mask diminished noradrenalin release by an imidazoline agonist (two opposite effects via stimulation and blockade of two separated receptors, i.e. physiological antagonism) and it can be no direct relation between imidazoline and α_2-adrenergic receptors in location site, so more investigations are needed to clarify the relation between α_2-adrenergic and imidazoline receptors.

Pretreatment with yohimbine didn't itself alter naloxone-induced jumping in comparison to control group. There are contrary reports about the effect of yohimbine on opioid withdrawal. It has been reported that the drug exacerbated the expression of morphine withdrawal syndrome in mice (17, 28) and human (10) or attenuated jumping behavior when it was used during the development of dependence (2, 20, 29). Another report showed no response for the drug (30). The contrary responses found by different investigators may be due to differences in the doses of drug used, in animals, in programs used in each study (before naloxone injection or during induction of morphine dependence) or be a reflection of α_2-adrenoceptor effects on presynaptic or postsynaptic sites of the adrenoceptor system.

Administration of clonidine, tizanidine and rilmenidine increased piloerection significantly. The mechanism of this paradoxical effect is not clear however; an increase in piloerection as a result of saline administration in morphine-dependent mice has also been reported (16).

Clonidine and tizanidine alleviated tremor significantly. It may be due to reduction in sympathetic tone by presynaptic α_2-adrenoceptor stimulation (31).

Clonidine and tizanidine also decreased diarrhea significantly, it may be due to a decrease in salt and water flux in to the lumen of intestine as a result of α_2-adrenoceptor activation in gastrointestinal (GI) tract (32), rilmenidine also decreased diarrhea significantly, which may suggest a role of imidazoline receptors in gastrointestinal tract.

In one part of our study, we supposed that if naloxone-induced opioid withdrawal signs are mediated at least partly through an increase in adrenergic activity in sites such as locus coeruleus, thus an α_2-adrenergic receptor blocker such as yohimbine can also induce morphine withdrawal signs when it is administered in morphine-dependent mice. Substitution of naloxone (5 mg/kg, i.p.) with yohimbine (4 mg/kg, i.p.) couldn't induce jumping in morphine-dependent mice and an increase in yohimbine dose (10 or 20 mg/kg) not only didn't induce jumping but also was lethal. Surprisingly yohimbine (2 or 4 mg/kg, i.p.) administration prior to naloxone injection in morphine-dependent mice was also lethal; it may be explained as such that adrenergic overactivity occurred during naloxone challenge may be exacerbated via prior blockade of auto-regulatory presynaptic α_2- adrenoceptors (17). Regarding to the yohimbine oral LD_{50} in mice which is 40 mg/kg (18), our results suggest that an investigation is needed to ascertain the safety of yohimbine administration in morphine-dependent subjects or patients who receive yohimbine co-treatment with naloxone or naltrexone.

Taken together, our results indicate that rilmenidine could alleviate some of the naloxone-induced withdrawal symptoms such as jumping and diarrhea. By considering its better compliance (lack of sedation, dry mouth, postural hypotension and rebound hypertension) compared to clonidine (15), it is notable to consider a potential indication for this drug in patients experiencing morphine withdrawal syndrome, therefore more experimental and clinical studies should be performed to evaluate rilmenidine potential as a substitute for clonidine in control of morphine withdrawal syndrome.

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