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European Journal of Pharmacology 473 (2003) 19–25
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Effects of imipramine on the expression and development of morphine dependence in mice

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Received 9 April 2003; received in revised form 21 May 2003; accepted 26 May 2003

Abstract

In the present study, the effects of imipramine and/or α-adrenoceptor agents on naloxone-induced jumping in morphine-dependent mice were examined. In the first set of experiments, the drugs were used before naloxone injection, to test their effects on the expression of jumping. Administration of imipramine (10–60 mg/kg) 15 min before naloxone increased the number of jumping in mice. Injection of the α_2-adrenoceptor agonist, clonidine (0.1 mg/kg), or α_1-adrenoceptor agonist, phenylephrine (4 mg/kg), themselves neither altered naloxone-induced jumping nor influenced the imipramine response. The α_2-adrenoceptor antagonist, yohimbine (4 mg/kg), itself but not the α_1-adrenoceptor antagonist, prazosin (1 mg/kg), increased jumping and decreased the imipramine effect.

In the second set of experiments, imipramine and/or the α-adrenoceptor drugs were injected during the development of morphine dependence. Imipramine (10–40 mg/kg) increased the development of dependence and increased jumping was seen. Clonidine did not influence the imipramine effect. Phenylephrine was lethal in combination with imipramine. Both yohimbine and prazosin decreased the effect of imipramine. Imipramine and phenylephrine but not clonidine, yohimbine or prazosin decreased locomotion. It is concluded an α_2-adrenoceptor mechanism may be involved in the influence of imipramine on the expression and development of naloxone-induced withdrawal signs in mice.

Keywords: Jumping; Imipramine; Morphine; Naloxone; Adrenoceptor agent; (Mouse)

1. Introduction

Chronic use of opioids may induce drug dependence (Weis et al., 1983) and behavioural reinforcing effects (Bilsky et al., 1992). Changes in catecholaminergic, serotonergic, cholinergic, γ-amino-butyric acid (GABA)-ergic and peptidergic transmission have been reported during chronic opiate administration. Central catecholamines seem to have an important role in the expression of the somatic signs of withdrawal and the abstinence syndrome of opioids (see Maldonado, 1997). One of the important anatomical areas that is thought to represent a site of origin of the opioid withdrawal syndrome is the locus coeruleus (Maldonado et al., 1992; Redmond and Krystal, 1984) which is rich in opioid receptors and α-adrenoceptors (Aghajanian, 1982). The activity of noradrenergic neurones in the locus coeruleus is inhibited by opioids (Aghajanian, 1978) and the abrupt withdrawal of opioids causes hyperactivity of the locus coeruleus in opioid-dependent animals (Aghajanian, 1978). The α-adrenoceptor mechanism has also been implicated in the expression of some components of withdrawal (see Maldonado, 1997). Tricyclic antidepressants, including imipramine, inhibit noradrenaline and serotonin reuptake (Maj et al., 1998), thus increasing the monoamines' levels and alter the opioid dependence processes. In the present study, the effects of imipramine and/or α-adrenoceptor agents on the expression and development of dependence on morphine, in mice, were investigated.

2. Materials and methods

2.1. Animals

Male NMRI mice (20–30 g) were housed in plastic cages in an animal room maintained at 22–25 °C on a 12-h dark cycle. Food and water were available at all

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doi:10.1016/S0014-2999(03)01913-7

times except during the experiments. Each animal was used once only and was killed immediately after the experiment.

2.2. Induction of dependence

The mice were rendered dependent on morphine, based on the method we used previously (Zarrindast and Farzin, 1996). Morphine sulfate was injected subcutaneously (s.c.) three times daily at 8, 12 and 16 h on the following dosage schedule. The first three doses were 50, 50 and 75 mg/kg, respectively. The higher dose of the third daily injection was aimed to minimize any overnight withdrawal. Morphine administration was carried out over a maximum of 3 days for any group of mice. A dose of 50 mg/kg of morphine sulfate was also injected on the 4th day (2 h before naloxone injection). Hyperactivity and the Straub tail effect were seen after morphine injections. Loss of weight (8–9%) and death (1–2%) were observed on chronic administration of morphine sulfate.

2.3. Naloxone-induced jumping

Groups of 8 mice were tested for the occurrence of jumping after their tenth injection of morphine on day 4. Two hours after the last dose of morphine (50 mg/kg), abstinence was precipitated by a subcutaneous (s.c.) injection of naloxone (2 mg/kg) and the animals were then placed individually in a Perspex observation cylinder (15 cm diameter, 50 cm height). The number of jumps was recorded immediately after the injection of naloxone over a 30-min period.

2.4. Measurement of locomotor activity

Locomotion was measured with an activity meter, Animex, Type S(LKB Farrad). Each animal was placed in a plastic cage for 15 min to acclimatize to the environment. Immediately after drug administration, the animals were returned to the cage for test trials lasting for a period of 45 min. Locomotor activity was recorded for a period of 0–15 and 15–45 min after drug injection.

2.5. Drugs

The following drugs were used: imipramine hydrochloride (Pars-Daru, Iran), morphine sulphate (Temad, Iran), naloxone hydrochloride ampoules (Tolidaru, Iran), phenylephrine hydrochloride, prazosin hydrochloride, clonidine hydrochloride and yohimbine (Sigma, Poole, UK). The drugs were dissolved in saline and were injected intraperitoneally (i.p.) in a volume of 10 ml/kg, except morphine and naloxone which were administered subcutaneously (s.c.). The control groups received saline. The doses of drugs used had been shown to be active in previous studies (Zarrindast et al., 1999, 2000). The experimental protocol was approved by the research committee of the school of pharmacy, Tehran University of Medical Sciences (Res No. 820P, 2 April 2000).

2.6. Drug treatment

The animals received 10 injections of morphine as described in Section 2.2. in order to develop dependence on morphine. The number of jumps induced by naloxone was compared to the number for mice that received 10 injections of saline instead of morphine. Drugs were injected either before naloxone administration (effect of drugs on the expression) or during the development of dependence on morphine (effect of drugs on the development).

2.6.1. Experiment 1

All the animals were rendered dependent on morphine. One group of animals received either saline (10 ml/kg) or different doses of imipramine (10–60 mg/kg), 15 min before naloxone injection, to test the effect of imipramine on the expression of withdrawal signs (Fig. 1A). The second group of animals received saline or different doses of imipramine (10–40 mg/kg) 30 min after the first and second doses of morphine on day 1 and day 2, during the development of dependence, in order to test the effect of imipramine on the development of morphine dependence (Fig. 1B).

2.6.2. Experiment 2

Effects of imipramine in the presence or absence of adrenoceptor agonists (clonidine and phenylephrine) on the expression or the development of naloxone-induced jumping in morphine-dependent animals. In one group of

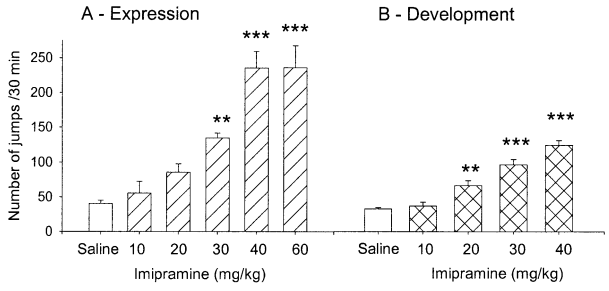

Fig. 1. (A) Effect of imipramine on the expression of naloxone-induced jumping in morphine-dependent mice. Mice were made dependent as described in the Methods section. All the dependent animals received naloxone (2 mg/kg) to induce jumping. The animals received saline or different doses of imipramine (10, 20, 30, 40 and 60 mg/kg) intraperitoneally (i.p.) 15 min before naloxone administration. (B) Effect of imipramine on the development of naloxone-induced jumping. The animals received saline or different doses of imipramine (10, 20, 30 and 40 mg/kg, i.p.) 30 min after the first and second daily doses of morphine, on days 1 and 2 (during the development of dependence on morphine). Naloxone (2 mg/kg) was used on day 4 to test jumping. Each group comprised 8 mice. Data are means ± S.E.M. $^{**}P<0.01$, $^{***}P<0.001$ different from the saline control group.

animals, imipramine was given 15 min, and the adrenoceptor agonists 30 min, before naloxone administration, on day 4, in order to examine the effect of the drugs on the expression of jumping in morphine-dependent animals (Fig. 2A). A second group of animals received different doses of imipramine (5, 10 and 20 mg/kg) with or without clonidine (0.01 mg/kg), during the development of dependence. Clonidine was injected 15 min, and imipramine 30 min, after the first and second doses of morphine on day 1 and day 2, in order to test the effect of the drugs on the development of dependence on morphine (Fig. 2B). Since the combination of even low-dose phenylephrine (0.25 mg/kg) with imipramine was lethal, no effect of their combination on morphine dependence can be shown.

2.6.3. Experiment 3

A group of animals received different doses of clonidine (0.01, 0.02 and 0.03 mg/kg) or saline, 15 min after the first and second doses of morphine on day 1 and 2, during the development of dependence, in order to test the effect of clonidine on the development of dependence on morphine (Fig. 3).

2.6.4. Experiment 4

Effects of imipramine, imipramine plus yohimbine, or plus prazosin, on the expression or development of naloxone-induced jumping. The first group of animals received imipramine, 15 min, and the α-adrenoceptor antagonists, 30 min, before naloxone administration, to test the effect of the drugs on the expression of jumping. The results are shown in Fig. 4A. The second group of animals received imipramine, imipramine plus yohimbine (4 mg/kg) or plus prazosin (1 mg/kg), during the development of dependence. Imipramine was injected 30 min, and the antagonists 15 min, after the first and second doses of morphine on days 1 and 2, during the develop-

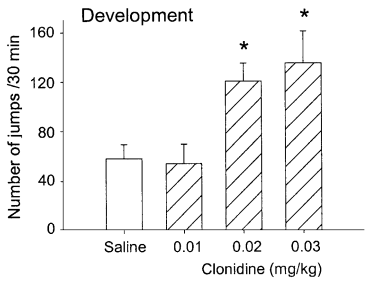

Fig. 3. Effects of clonidine on the development of morphine dependence in mice. The mice received different doses of clonidine (0.01, 0.02 and 0.03 mg/kg) during the development of dependence on morphine. Clonidine was injected 15 min after the first and second dose of morphine daily, on day 1 and 2 (during the development of dependency to morphine). Naloxone (2 mg/kg) was used on day 4, to test jumping. Each group comprised 8 mice. Data are means ± S.E.M. *$P<0.05$ different from respective imipramine group.

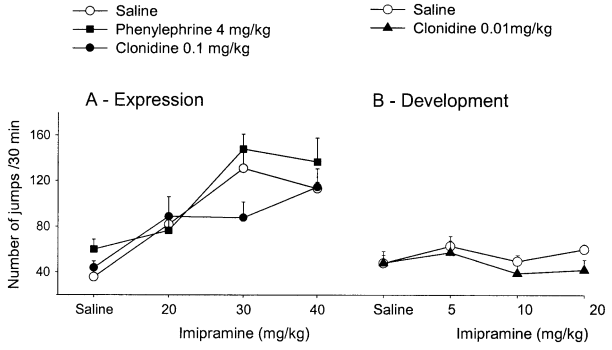

Fig. 2. (A) Effect of clonidine and phenylephrine on the influence of imipramine on the expression of naloxone-induced jumping in morphine-dependent mice. Mice were made dependent as described in Section 2. All the dependent animals received naloxone (2 mg/kg) to induce jumping. A group of animals received saline or different doses of imipramine (20–40 mg/kg). Two other groups of animals received saline or imipramine plus clonidine (0.1 mg/kg) or plus phenylephrine (4 mg/kg). Saline imipramine was administered 15 min, and clonidine or phenylephrine 30 min, before naloxone injection. (B) Effect of imipramine, in the presence or absence of clonidine, on the development of morphine dependence in mice. A group of mice received saline or different doses of imipramine (5, 10 and 20 mg/kg, i.p.). Two other groups of animals received saline or imipramine plus clonidine (0.01 mg/kg) during the development of dependence on morphine. Saline or imipramine was injected 30 min, and clonidine 15 min, after the first and second doses of morphine daily, on days 1 and 2 (during the development of dependence on morphine). Naloxone (2 mg/kg) was used on day 4 to test jumping. Each group comprised 8 mice. Data are means ± S.E.M.

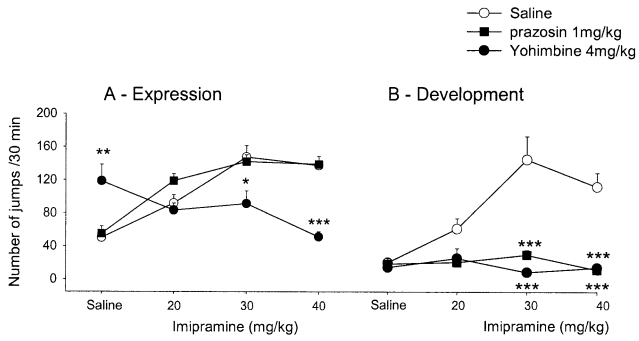

Fig. 4. (A) Effect of yohimbine or prazosin on the influence of imipramine on the expression of naloxone-induced jumping in morphine-dependent mice. All the dependent animals received naloxone (2 mg/kg) to induce jumping. One group of animals received saline or different doses of imipramine (20, 30 and 40 mg/kg). Two other groups of animals received saline or imipramine plus yohimbine (4 mg/kg) or plus prazosin (1 mg/kg). Saline or imipramine was administered 15 min, and yohimbine or prazosin 30 min, before naloxone injection. (B) Effects of imipramine in the presence or absence of yohimbine or prazosin on the development of morphine dependence in mice. A group of dependent mice received different doses of saline or imipramine (20, 30 and 40 mg/kg, i.p.). Two other groups of animals received saline or imipramine plus yohimbine (4 mg/kg) or prazosin (1 mg/kg). Imipramine was injected 30 min, and yohimbine or prazosin 15 min, after the first and second doses of morphine daily, on days 1 and 2 (during the development of dependence on morphine). Naloxone (2 mg/kg) was used on day 4 to test jumping. Each group comprised 8 mice. Data are means ± S.E.M. *$P<0.05$, **$P<0.01$, ***$P<0.0001$ different from respective imipramine control group.

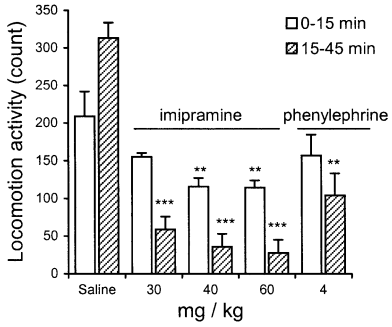

Fig. 5. Effects of imipramine and phenylephrine on locomotor activity in mice. The animals received saline (10 ml/kg), imipramine (30, 40 and 60 mg/kg) or phenylephrine (4 mg/kg). Locomotor activity was measured from 0–15 and 15–45 min after the drugs administration. Each group comprised 8 mice. Data are means ± S.E.M. $^{**}P<0.01$, $^{***}P<0.001$ different from respective saline control group.

ment of dependence. Naloxone was used on day 4, to test jumping (Fig. 4B).

2.6.5. Experiment 5

Effects of imipramine, phenylephrine, clonidine, yohimbine or prazosin on locomotor activity. The animals received saline (10 ml/kg), imipramine (30, 40 and 60 mg/kg), phenylephrine (4 mg/kg), clonidine (1 mg/kg), yohimbine (4 mg/kg) or prazosin (1 mg/kg). Locomotion was recorded for 0–15 and 15–45 min after drug injection. The effects of imipramine and phenylephrine are shown in Fig. 5.

2.7. Statistical analysis

Analyses of variance (ANOVAs) followed by Newman–Keuls test were used for analysis of the data. Differences between means were considered statistically significant if $P<0.05$. Each point is the Mean ± S.E.M. for 8 mice.

3. Results

3.1. Naloxone-induced withdrawal jumping in morphine-dependent mice

The mice were divided randomly into two groups. One group received morphine (as described in Section 2) to induce dependence. The next group received saline (10 ml/kg) instead of morphine subcutaneously (s.c.). Naloxone (2 mg/kg, s.c.) increased the number of jumps (58.1 ± 11.4; $N=8$) in morphine-dependent mice as compared to jumps (0.2 ± 0.2; $N=8$, $P<0.0001$) in non-dependent mice. The results showed that naloxone can induce jumping in morphine-dependent mice. We considered jumping behaviour as the sign of abstinence for further experiments in our study. Hyperactivity and the Straub-tail reaction were seen after morphine injections. Loss of weight (8–9%) and death (1–2%) occurred on chronic administration of morphine sulfate.

3.2. Effect of imipramine on the expression or development of naloxone-induced jumping behaviour in morphine-dependent mice

Dose–response for imipramine on the expression of naloxone-induced jumping (Fig. 1A). All animals received morphine (s.c.) three times daily for 3 days, in order to induce dependence on morphine as described earlier in Section 2. Different doses of imipramine were injected 15 min before naloxone on day 4, and then the number of jumps was recorded. The intraperitoneal (i.p.) administration of imipramine (10, 20, 30, 40 and 60 mg/kg), before naloxone increased jumping [one-way ANOVA, $F(5,42)=22.2$, $P<0.0001$] in morphine-dependent animals. The maximum effect of imipramine was obtained with 40 mg/kg.

Effect of imipramine on the development of dependence (Fig. 1B). All animals received morphine (s.c.) three times daily for 3 days, in order to induce dependence on morphine as described earlier. Different doses of imipramine were injected 30 min after morphine administration, for the first and second doses of morphine on days 1 and 2. The number of jumps was recorded on day 4. The intraperitoneal (i.p.) administration of imipramine (10, 20, 30 and 40 mg/kg) increased naloxone-induced jumping [one-way ANOVA, $F(4,35)=41.6$, $P<0.0001$] in morphine-dependent animals. The maximum effect of imipramine was obtained with 40 mg/kg.

3.3. Effects of imipramine with or without α-adrenoceptor agonists on the expression and development of naloxone-induced jumping behaviour in morphine-dependent mice

Fig. 2A shows the effect of imipramine with or without the α_2-adrenoceptor agonist, clonidine, or with the α_1-adrenoceptor agonist, phenylephrine, on the expression of naloxone-induced jumping. The animals were rendered dependent as described earlier. Clonidine and phenylephrine were injected 15 min before imipramine and imipramine 15 min prior to naloxone on day 4, in morphine-dependent animals. Two-way ANOVA showed that the administration of different doses of imipramine (20, 30 and 40 mg/kg) did not interact either clonidine (0.1 mg/kg, i.p.) [$F(3,56)=2.1$, $P>0.05$] or phenylephrine (4 mg/kg, i.p.) [$F(3,56)=0.6$, $P>0.05$]. However, post hoc analysis showed that imipramine itself increased jumping.

Fig. 2B indicates the effect of imipramine with and without clonidine on the development of morphine dependence. Two-way ANOVA indicates that the administration of different doses of imipramine (5, 10, and 20 mg/kg, i.p.) with clonidine (0.01 mg/kg, i.p.) during the development of morphine-dependence, did not led to an interaction [$F(3,56)=0.55$, $P>0.05$]. Clonidine itself, when used during the development (in the absence of morphine) in-

creased jumping [one-way ANOVA; $F(3,26)=5.6$, $P<0.01$] (Fig. 3).

Since the combination of even a low dose of phenylephrine (0.25 mg/kg) with imipramine was lethal, no effect of their combination on morphine dependence can be shown.

3.4. Effects of imipramine with or without α-adrenoceptor antagonists on the expression and development of naloxone-induced jumping behaviour in morphine-dependent mice

Fig. 4A shows the effect of imipramine with or without the α_2-adrenoceptor antagonist, yohimbine, or the α_1-adrenoceptor antagonist, prazosin on the expression of naloxone-induced jumping. Two-way ANOVA showed that the administration of different doses of imipramine (20, 30 and 40 mg/kg) with yohimbine (4 mg/kg, i.p.) led to interactions [$F(3,56)=14.5$, $P<0.0001$]. Further analysis indicates that yohimbine itself increased the expression of naloxone-induced jumping, but reduced the imipramine response. Prazosin did not show interactions with the imipramine response [$F(3,56)=1.1$, $P>0.05$].

Fig. 4B shows the effect of yohimbine and prazosin in the presence or absence of imipramine when administered during the development of morphine dependence. Two-way ANOVA showed that the injection of different doses of imipramine (20, 30 and 40 mg/kg) with yohimbine led to interactions [$F(3,56)=10.4$, $P<0.0001$]. Imipramine also interacted with prazosin [$F(3,56)=7.7$, $P<0.0001$]. Further analysis showed that neither yohimbine nor prazosin themselves induced response, but the combination of both antagonists reduced the imipramine effect.

3.5. Effects of drugs on locomotion

Fig. 5 shows the effect of imipramine and phenylephrine on locomotor activity.

One-way ANOVA showed that the administration of different doses of imipramine (30, 40 and 60 mg/kg) and phenylephrine (4 mg/kg) 0–15 min [$F(4,30)=6.0$, $P<0.01$] and 15–45 min after drug administration [$F(4,30)=56.6$, $P<0.0001$], but not clonidine (0.01 mg/kg), yohimbine (4 mg/kg) or prazosin (1 mg/kg) decreased locomotion.

4. Discussion

Several neurotransmitters including serotonin, dopamine, GABA, adenosine, cholecystokinin and aspartate seem to be involved in morphine tolerance and dependence (Bhargava, 1994; Bourin, 1999). Several studies have shown that the noradrenergic and that the opioid systems interact in a complex manner and the interactions between the two systems are often reversible with opioid antagonists (see Hughes et al., 1996). The tricyclic antidepressant, imipramine, induces analgesia and increases morphine withdrawal signs (Baraldi et al., 1983). It also inhibits the reuptake of monoamines in the brain of both animals and humans (Maj et al., 1998). The fundamental role of α-adrenoceptors in the morphine withdrawal syndrome is not quite clear. In the present study, the effect of imipramine and/or adrenoceptor agents on the expression and development of dependence on morphine in mice were studied.

4.1. Part I. Effect of drugs on the expression of naloxone-induced jumping

Different doses of imipramine, when administered 15 min before naloxone in morphine-dependent mice, increased the expression of naloxone-induced jumping. The response to the drug was dose-dependent. The data may indicate that imipramine influences the expression of jumping. Thus adrenergic and/or serotonergic mechanism(s) may be implicated in the imipramine effect. Our present results show that administration of an α_2-adrenoceptor antagonist, yohimbine, but not of an α_1-adrenoceptor antagonist, prazosin, 15 min before imipramine injection, decreases the imipramine effect on the expression of jumping. One may propose that α_2-adrenoceptors are somehow involved in the imipramine response.

To clarify the involvement of α_2-adrenergic mechanims in the imipramine effect on the expression of naloxone-induced jumping in mice, further experiments with α_2-adrenergic agents were undertaken. The data showed that clonidine, which is an α_2-adrenoceptor agonist, did not itself alter jumping nor did it alter the effect of imipramine during the expression of jumping. In agreement with our data there is a report showing that no effect on clonidine jumping (Berthold et al., 1989). However, a report indicates that clonidine potentiates the expression of naloxone-induced jumping in morphine-dependent rats (Van der Laan, 1985). Furthermore, there are reports indicating that clonidine, given intracerebroventricularly at pmol–nmol doses, decreased the incidence of morphine withdrawal syndrome in rats and mice, while intracerebroventricular injection of yohimbine prevented the clonidine effect (Garzón and Sánchez-Blázquez, 1992). The data obtained by other investigators also indicate that low doses of clonidine ameliorate the withdrawal syndrome through stimulation of α_2-adrenoceptors, while yohimbine exacerbated withdrawal in rats (Dwoskin et al., 1983). Some data also indicate that yohimbine attenuates jumping behaviour (Berthold et al., 1989), whereas others found no response to the drug (Van der Laan, 1987). The contradictory results from different investigators may be due to different doses of the drug, animals or a reflection of α_2-adrenoceptor agents' effect on presynaptic or postsynaptic sites of the adrenergic system.

The present results also showed that the α_2-adrenoceptor antagonist, yohimbine, itself increased the expression of naloxone-induced jumping. This response to yohimbine may be due either to the inhibition of serotonin receptors or to the blockade of presynaptic α_2-adrenoceptors which may in turn release noradrenaline. This explanation may be

supported by the results showing that yohimbine potentiates naloxone-induced jumping in mice (Sharif and El Kadi, 1996) and that it elicits objective and subjective opioid withdrawal symptoms with elevated craving for opioid drugs in opioid-dependent patients (Stine et al., 2002).

In the present study, the α_1-adrenoceptor agonist, phenylephrine, and the α_1-adrenoceptor antagonist, prazosin, neither altered the imipramine effect on the expression of jumping, nor themselves influenced jumping, which may be in agreement with findings of others indicating the failure of prazosin to elicit any response in this respect (Sharif and El Kadi, 1996). However, there is a report indicating that α_1-adrenoceptors may be involved in the morphine withdrawal symptoms (see Maldonado, 1997) and moreover, it has been shown that prazosin decreases the incidence of some of the somatic signs of opiate withdrawal (Van der Laan, 1985). The involvement of adrenoceptors in the appearance of symptoms is also demonstrated by the positive effects of prazosin, which prevents acetylcholine facilitation by naloxone (Beani et al., 1989). Whether other mechanisms are involved in the prazosin response seen in the present study needs to be examined. It should be noted that imipramine and phenylephrine, but not clonidine, yohimbine or prazosin, decreased locomotor activity. Therefore, the jumping induced by imipramine may possibly be influenced by this effect of the drug.

4.2. Part 2. Effect of drugs on the development of morphine dependence

Imipramine administration during the development of dependence on morphine, increased naloxone-induced jumping. However, the drug itself (without morphine administration) did not induce jumping (data not shown). Administration of imipramine, during the development of dependence, has been shown in rats, to intensify the morphine withdrawal syndrome precipitated by naloxone (Baraldi et al., 1983), which may support the present results. Since it has been shown that chronic administration of imipramine increases opioid binding sites in the brain (Baraldi et al., 1983), one explanation may be potentiation of the morphine effect through this mechanism. The present data showed that pretreatment of animals with yohimbine, during the development of morphine dependence, decreased the imipramine response. There is also a possibility that α_2-adrenoceptors play a role in the opioid analgesic effect (Gray et al., 1999). Therefore, imipramine may increase the morphine response through inhibition of monoamine reuptake and influence the adrenoceptor mechanism(s). The concomitant chronic administration of yohimbine with morphine (during the development of morphine dependence) did not elicit any response, although other investigators (El-Kadi and Sharif, 1997; Iglesias et al., 1992; Taylor et al., 1991) indicate that yohimbine may decrease morphine dependence. Moreover, the α_2-adrenergic system has also been shown to be important in the development of opioid physical dependence (see Iglesias et al., 1992). Thus the involvement of an α_2-adrenoceptor mechanism in the imipramine-increased morphine dependence in mice seems likely. However, while drugs usually induce opposite effects on expression and development of dependence, imipramine elicits similar effects on both phenomena. In addition to an increase in opiate binding sites on chronic administration of imipramine (Baraldi et al., 1983), the latter induces downregulation of α_2-adrenoceptors (Jiménez-Rivera et al., 1996). Although the drug's effect on expression may be partly mediated through an α_2-adrenoceptor mechanism, its effect on dependence seems to be achieved by different mechanisms.

The α_1-adrenoceptor agent, prazosin, also reduced the imipramine effect on the development of morphine dependence in the present study. Prolonged treatment with imipramine has been shown to markedly increase the basal activity of protein kinase C in the membrane fractions of the frontal cortex and hippocampus (Szmigielsi and Górska, 1997), which may account for the prazosin response. Furthermore, the drug has been shown to block α_{2B}-adrenoceptors (Harrison et al., 1991). Therefore, the involvement of α_{2B}-adrenoceptors in the prazosin response cannot be excluded.

In the present study, a combination of lower doses of imipramine and clonidine was used during the development of morphine dependence, in order to examine the potentiation of their effect. Our data showed that clonidine, when used during the development of dependence on morphine, did not alter the imipramine-induced increase in jumping in morphine-dependent animals. Higher doses of clonidine, in combination with imipramine, had a lethal effect. Higher doses of clonidine alone, when administered during the development of dependence, increased naloxone-induced jumping.

Phenylephrine was also administered during the development of dependence. The combination of imipramine with even low doses of phenylephrine (0.25 mg/g) was lethal, and therefore no data can be shown in this respect.

Acknowledgements

The authors wish to thank Dr. Touraj Nayer-Nouri for his assistance in preparing this manuscript.

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