Available online at www.sciencedirect.com
SCIENCE DIRECT
Pharmacology, Biochemistry and Behavior 82 (2005) 601 – 607
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Morphine withdrawal syndrome: Involvement of the dopaminergic system in prepubertal male and female mice

Silvina L. Diaz, Alma K. Kemmling, Modesto C. Rubio, Graciela N. Balerio *

Instituto de Investigaciones Farmacológicas (CONICET), Buenos Aires, Argentina
Cátedra de Farmacología, Facultad de Farmacia y Bioquímica (Universidad de Buenos Aires), Junín 956, 5° floor, Buenos Aires (1113AAD), Argentina

Received 20 May 2005; received in revised form 5 October 2005; accepted 20 October 2005
Available online 1 December 2005

Abstract

Morphine (MOR) withdrawal signs are more marked in males than in females. Considering that the influence of the dopaminergic system on these differences is unclear, we analyzed dopamine (DA) and dihydroxyphenylacetic-acid (DOPAC) brain levels during naloxone (NAL)-precipitated withdrawal as well as the involvement of D_1 and D_2 receptors in the expression of MOR withdrawal in either sex. Prepubertal Swiss-Webster mice received MOR (2 mg/kg, i.p.) twice daily for 9 days. On the tenth day, dependent animals received NAL (6 mg/kg, i.p.) after MOR and were sacrificed 30 min later. DA and DOPAC concentrations were determined in different brain areas using HPLC with electrochemical detection. Other pool of mice received either a D_1 (SCH 23390; 0.2 mg/kg, i.p.) or D_2 (raclopride; 0.3 mg/kg, i.p.) receptor antagonist before NAL and withdrawal signs were evaluated. DA and DOPAC levels only decreased in striatum and cortex of withdrawn males. Conversely, both DA receptor antagonists decreased the expression of MOR withdrawal signs in either sex. The neurochemical sex differences described here could partially explain the behavioral sex differences observed during MOR withdrawal. Additionally, SCH-23390 and raclopride effects suggest an important role of both DA receptors in the expression of MOR withdrawal in males and females.

Keywords: Naloxone-precipitated withdrawal; Mice; Striatum; Frontal cortex; Dopamine; Sex differences; Raclopride; SCH 23390

1. Introduction

Several studies have demonstrated sex-related differences in many pharmacological properties of morphine (MOR): antinociception (Candido et al., 1992; Cicero et al., 1997), tolerance to analgesia (Kest et al., 2000) and conditioned analgesia (Stock et al., 2001). These studies stated that female rodents are less sensitive to MOR properties than males, but the mechanisms of this sex dimorphism still remain uncertain. In this context, we have demonstrated that female prepubertal mice were less prone to develop the signs of MOR withdrawal syndrome than males (Diaz et al., 2001) which is in agreement with previous results (Craft et al., 1999). Additionally, we have also demonstrated that an increase in μ-opioid receptor density occurred in male mice during the MOR withdrawal syndrome, but not in females (Diaz et al., 2004).

The development of opiate dependence as well as the expression of MOR withdrawal syndrome are both due to processes of homologous regulation affecting the endogenous opioid system and heterologous regulation that affect other neurotransmitter systems (Koob and Bloom, 1988). Previous studies have revealed that dopamine (DA) neurotransmission plays an important role in the MOR withdrawal syndrome (Acquas and Di Chiara, 1992; Diana et al., 1995; Diaz et al., 2003), but all these experiments have been performed in male animals, i.e., no data is available from females. In addition, there is a paucity of data about sex differences on brain monoamine concentrations during chronic MOR treatment and particularly, no information is available from mice. Brain areas such as striatum and frontal cortex have been related with the MOR withdrawal syndrome (Bassareo et al., 1995; Espejo et al., 2001; Diaz et al., 2003, 2004), but further studies would be necessary to explain the involvement of

* Corresponding author. Cátedra de Farmacología, Facultad de Farmacia y Bioquímica (Universidad de Buenos Aires), Junín 956, 5° floor, Buenos Aires (1113AAD), Argentina. Tel.: +54 11 4961 5949; fax: +54 11 4963 8593.
E-mail address: gbalerio@ffyb.uba.ar (G.N. Balerio).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.10.012

these areas in the expression of the MOR withdrawal syndrome.

In previous studies, DA agonists have been found able to potentiate several MOR withdrawal-induced signs in rodents (Gianutsos et al., 1976; Kantak and Miczek, 1988; Tidey and Miczek, 1992). Additionally, D_1 and D_2 receptor antagonists have been found to decrease the expression of MOR withdrawal-induced aggression in male mice (Rodriguez-Arias et al., 1999) as well as other signs in MOR withdrawn rats (Funada and Shippenberg, 1996; El-Kadi and Sharif, 1998; Zarrindast et al., 2002). Even though previous data suggest that the mesolimbic dopaminergic neurotransmission mediates several behavioral effects of opiates, the differential role of D_1 and D_2 receptors during MOR withdrawal remains unclear and has not been explored in female mice.

Since we have demonstrated sex-related behavioral differences in the expression of the MOR-withdrawal syndrome (Diaz et al., 2001) and taking into account the decreased striatal and cortical DA concentrations observed in MOR withdrawn male mice (Diaz et al., 2003), the aim of the present study was to analyze and compare striatal, cortical and hippocampal DA and dihydroxyphenylacetic acid (DOPAC) brain concentrations in prepubertal male and female mice during the MOR withdrawal syndrome. Another aim of this study was to evaluate the effect of the pretreatment of the selective D_1 (SCH 23390) and D_2 (raclopride) receptor antagonists during MOR withdrawal in either sex.

2. Methods

2.1. Subjects

Prepubertal Swiss-Webster male and female albino mice were obtained from our breeding colony of the Department of Pharmacology (Faculty of Pharmacy and Biochemistry) of the University of Buenos Aires. Experiments were performed on naïve prepubertal (indicated by vaginal smears) male and female mice weighing 20 g at the beginning of the treatment. Animals were housed in groups of five under conditions of constant temperature (22 ± 2 °C) and relative humidity (55 ± 15%), according to local regulations (SENASA). Mice were housed under a standard 12 h light/dark cycle (lights on 08:00 a.m.) with free access to food and water up to the beginning of the experiments. Experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs

Morphine hydrochloride (Chemotecnia Sintyal, Buenos Aires, Argentina), naloxone (Endo Laboratories, USA), SCH 23390 (RBI, USA) and raclopride (SIGMA-RBI, USA) were used to develop this study. All the drugs were dissolved in isotonic (NaCl 0.9%) saline solution and the doses were referred to the salt form. All the drugs were injected intraperitoneally (i.p.) in a volume of 0.1 ml/10 g of body weight.

2.3. Induction of MOR dependence

Mice were rendered dependent on MOR (as it was described in Diaz et al., 2001) by intraperitoneally (i.p.) injection of MOR (2 mg/kg), twice daily (10:00 h and 22:00 h), for 9 consecutive days.

2.4. Neurochemical studies

2.4.1. Drug treatment

On the day of the experiment (tenth day), dependent mice received the last dose of MOR only at 10:00 h and then were randomly divided into two groups ($n = 5–6$) as follows:

- **Dependence**: 60 min after the last dose of MOR, mice received saline (SAL).
- **MOR withdrawal**: 60 min after the last dose of MOR, mice received naloxone (NAL; 6 mg/kg). The dose of NAL used to precipitate the withdrawal syndrome in the present study was the same reported in our previous studies which did not induce a withdrawal syndrome in non-dependent mice, but did induce a full withdrawal syndrome in MOR-dependent mice (Diaz et al., 2001).

The control groups received SAL, twice daily for 9 consecutive days. On the tenth day animals received the last injection of SAL at 10:00 h and were divided into two control groups ($n = 5–6$):

- **SAL control**: 60 min after the last injection of SAL, mice received SAL.
- **NAL control**: 60 min after the last injection of SAL, mice received NAL.

2.4.2. Electrochemical detection

High Performance Liquid Cromatography (HPLC)-coupled electrochemical detection (Heikkila et al., 1984) of DA and DOPAC was achieved using a Varian 5000 liquid chromatograph coupled to an electrochemical detector (BAS LC-4C). Ten minutes after the last injection on the tenth day, brains were collected and striatum, cortex and hippocampus were dissected, weighed, homogenized, and deproteinized in 0.2 N perchloric acid (1/20 mg/ml). Homogenates were centrifuged and the supernatants were injected (50 μl) onto a 12.5 cm × 4 mm Nova-Pak C18 reverse phase column (Waters) developed in 250 ml of mobile phase (0.076 M $NaH_2PO_4 \cdot H_2O$; 5.24 ml/L PICB8, 0.99 mM EDTA, 6% methanol) 1.3 ml/min. The electrode potential was set at +0.7 V. Peak heights were measured by DATA Jet Integrator (Spectra-Physics) and quantified based on standard curves using Excel.

2.5. Behavioral studies

2.5.1. Drug treatment

The day of the experiment (tenth day), dependent mice received the last dose of MOR only at 10:00 h and

then were randomly divided into the following groups ($n=9–14$):

- **MOR withdrawal**: 30 and 60 min after the last dose of MOR, mice received SAL and NAL, respectively.
- **MOR withdrawal+SCH 23390**: 30 and 60 min after the last dose of MOR, mice received SCH 23390 (0.2 mg/kg) and NAL, respectively.
- **MOR withdrawal+raclopride**: 30 and 60 min after the last dose of MOR, mice received raclopride (0.3 mg/kg) and NAL, respectively.

The doses of the DA antagonists were selected after trying increasing doses on naïve mice by open field method in order to rule out a hypolocomotor effect.

The control groups received SAL, twice daily for 9 consecutive days. The tenth day animals received the last injection of SAL at 10:00 h and were divided into the following groups ($n=9–14$):

- **SAL control**: 30 and 60 min after the last injection of SAL, mice received SAL.
- **SCH 23390 control**: 30 and 60 min after the last injection of SAL, mice received SCH 23390 (0.2 mg/kg) and SAL, respectively.
- **Raclopride control**: 30 and 60 min after the last injection of SAL, mice received raclopride (0.3 mg/kg) and SAL, respectively.

2.5.2. Apparatus and procedure

Experiments were conducted in an open field, an apparatus consisting of a black Plexiglass box ($33\times43\times16$ cm) with a painted grid in the white floor dividing it into equal squares (7×7 cm) (Diaz et al., 2001). Each animal was challenged once. Experiments were performed at the same hour (between 11.00 and 16.00 h) and same place, with similar temperature and light conditions.

After the last injection of NAL or SAL, animals were placed in one of the corners of the box and the number of withdrawal signs was recorded over a 30-min period by one observer who was blind to the treatment conditions.

2.5.3. Measurements

After the different treatments, animals were challenged for 30 min in the open field. Based in our previous report in which behavioral sex differences have been demonstrated (Diaz et al., 2001), in the present study we have only measured the signs which were modified by the MOR withdrawal state. Two types of signs were measured during MOR abstinence. The number of sniffing, wet dog shakes and jumps were counted. Diarrhoea was evaluated with a point being given for the presence of this sign. In order to summarize the results obtained from the different observations, a global withdrawal score was calculated individually for each mouse. To obtain this global value and to give all the signs a proportional weighting, the score obtained from each sign was multiplied by a constant as previously reported (Maldonado et al., 1996) as follows: sniffing $\times0.5$; wet dog shakes $\times1$; jumping $\times0.8$; diarrhoea $\times1.5$.

2.5.4. Statistical analysis

To determine differences between the experimental groups for both neurochemical and behavioral studies, two-way analysis of variance (ANOVA) with sex and treatment as the main factors was used after verifying normality of distribution and homoscedasticity by Shapiro-Wilks test and Levene's test, respectively. With a statistically significant interaction, simple main effects were analyzed, and Newman-Keuls tests were used for post hoc comparisons. In all cases, $p<0.05$ was considered statistically significant.

3. Results

3.1. Neurochemical studies

3.1.1. Striatum

Fig. 1(A and B) summarizes the changes in DA and DOPAC levels determined in striatum. Two-way ANOVA revealed a significant interaction for DA levels, $F(3, 38)=3.92$, $p<0.05$ as well as for DOPAC levels $F(3, 33)=4.48$, $p<0.01$, a main effect of sex for DA levels, $F(1, 38)=4.65$, $p<0.05$ as well as for DOPAC levels $F(1, 33)=10.07$, $p<0.01$ and a main effect for treatment only for DOPAC levels $F(1, 33)=6.44$, $p<0.01$. Simple main effects revealed a significant effect of treatment in male mice for DA concentrations, $F(3, 38)=5.42$, $p<0.001$ and also for DOPAC concentrations, $F(3, 33)=6.52$, $p<0.001$. Post hoc analysis indicated that DA and DOPAC levels in MOR-withdrawn male mice decreased 40% and 67%, respectively, ($p<0.05$) compared with their SAL control groups. On the contrary, simple main effects analysis indicated no differences between female experimental groups neither for DA, $F(3, 38)=1.25$, NS nor for DOPAC, $F(3, 33)=2.66$, NS. The simple main effects analysis also revealed a significant effect of sex between withdrawal groups for DA concentrations, $F(1, 38)=14.5$, $p<0.001$ and also for DOPAC concentrations, $F(1, 33)=11.66$, $p<0.01$. No sex differences were found for the rest of the treatments.

3.1.2. Cortex

Fig. 1(C and D) summarizes the changes in DA and DOPAC levels determined in frontal cortex. Two-way ANOVA revealed no significant interaction for DA levels, $F(3, 34)=1.77$, NS, nor effect of sex $F(1, 34)=2.77$, NS, and a significant effect of treatment $F(3, 34)=7.46$, $p<0.001$. Post hoc analysis indicated that DA levels in MOR-withdrawn male mice decreased 67% ($p<0.05$) with respect to its SAL control group. In opposition, no differences were found between female experimental groups for DA, $F(3, 20)=3.44$, NS.

Two-way ANOVA revealed no significant interaction for DOPAC levels, $F(3, 37)=0.59$, NS, no effect of treatment, $F(3, 37)=2.33$, NS or sex, $F(1, 37)=2.28$, NS.

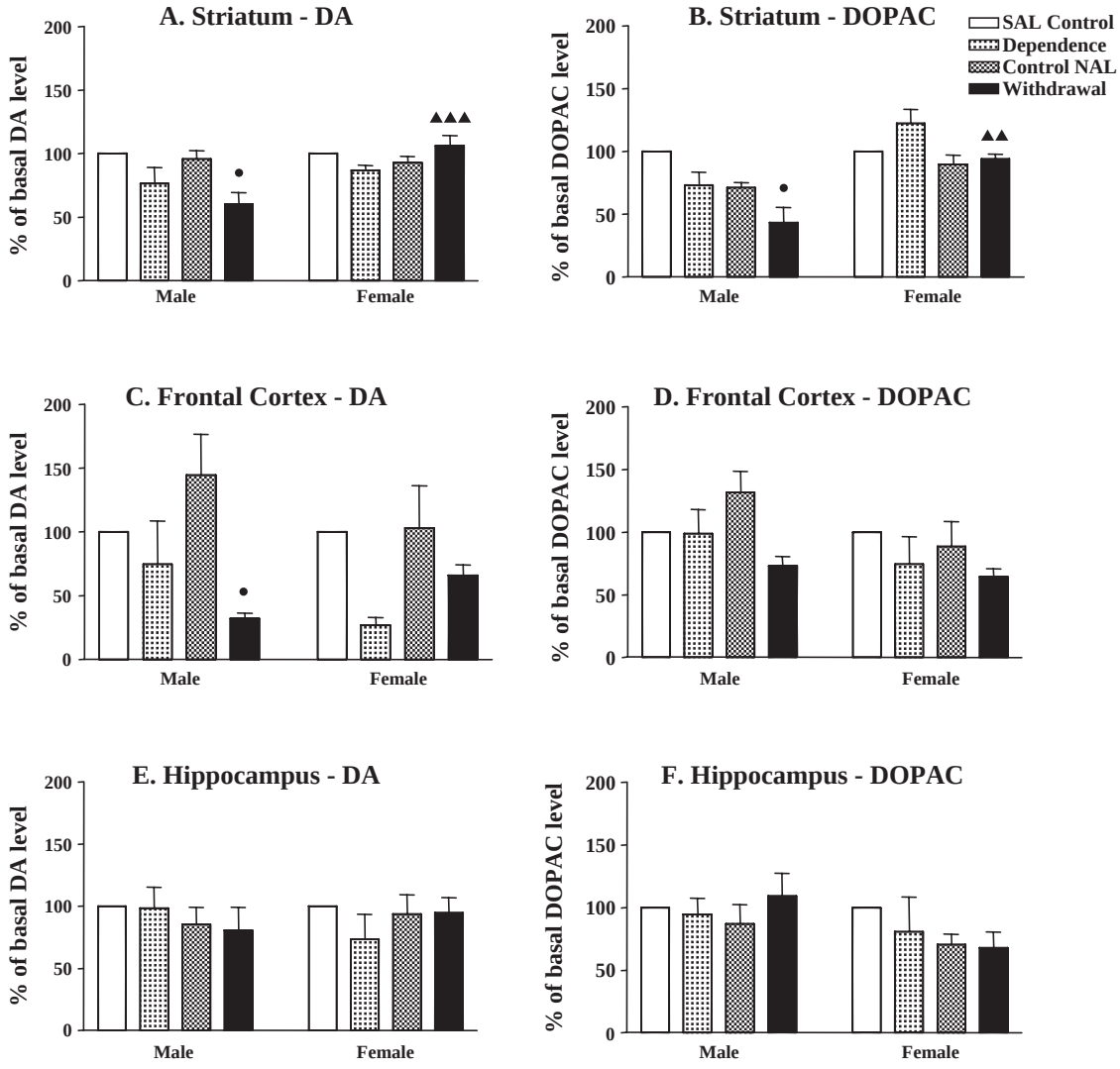

Fig. 1. Changes in the endogenous brain levels of DA and DOPAC in mice brain areas. Data represent the values (mean ± S.E.M.) obtained in male and female experimental groups, expressed as a percentage of their SAL control values in order to compare relative changes in either sex. ● $p<0.05$ with respect to the male SAL control group; ▲▲ $p<0.01$; ▲▲▲ $p<0.001$ with respect to the male MOR withdrawal group (ANOVA, Newman Keuls test). Values expressed as pmol/mg of tissue for SAL controls groups were as follows: Striatum DA level in males and females: 25.8 ± 5.8 and 25.4 ± 4.7, respectively; Striatum DOPAC level in males and females: 8.8 ± 1.2 and 7.8 ± 1.6, respectively. Cortical DA level in males and females: 0.32 ± 0.10 and 0.26 ± 0.09, respectively. Cortical DOPAC level in males and females: 0.47 ± 0.08 and 0.48 ± 0.19, respectively. Hippocampal DA level in males and females: 0.11 ± 0.02 and 0.15 ± 0.05, respectively. Hippocampal DOPAC level in males and females: 0.22 ± 0.10 and 0.28 ± 0.11, respectively.

3.1.3. Hippocampus

Fig. 1(E and F) summarizes DA and DOPAC levels determined in hippocampus. Two-way ANOVA revealed no significant interaction for DA levels, $F(3, 35)=0.91$, NS, no effect of treatment, $F(3, 35)=0.31$, NS or sex, $F(1, 35)=7.51$, NS. The same analysis applied to DOPAC levels revealed neither a significant interaction, $F(3, 32)=0.50$, NS, no effect for treatment, $F(3, 32)=0.40$, NS or sex, $F(1, 32)=0.06$, NS.

3.2. Behavioral studies

Two-way ANOVA revealed a significant interaction, $F(5, 123)=2.89$, $p<0.01$, a main effect of treatment $F(5, 123)=24.27$, $p<0.001$ and no effect of sex $F(1, 123)=2.77$, NS for the global score. Simple main effect revealed a significant effect of treatment in males $F(5, 123)=24.15$, $p<0.001$ and in females $F(5, 123)=5.43$, $p<0.001$. Post hoc analysis indicated that the global score of MOR-withdrawn male and female mice were significant higher ($p<0.001$) than the global score of their respective SAL control groups (Fig. 2). Additionally, the pretreatment with SCH 23390 or raclopride significantly decreased ($p<0.001$) the global score of the MOR withdrawn male and female mice. The simple main effect analysis also revealed a significant effect of sex between withdrawal groups $F(1, 123)=15.37$, $p<0.001$. No sex differences were found for the rest of the treatments.

4. Discussion

Two main findings emerge from the present study. First, changes in the striatal and cortical dopaminergic system appear to be involved in the expression of the MOR withdrawal

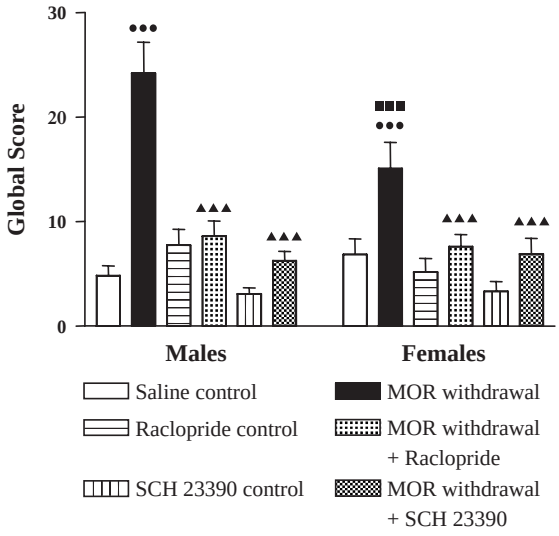

Fig. 2. Attenuation of the severity of NAL-precipitated MOR withdrawal syndrome by acute raclopride or SCH 23390. Raclopride (0.3 mg/kg, i.p.) or saline, and SCH 23390 (0.2 mg/kg, i.p.) or saline were administered 30 min before withdrawal. A global score was calculated for each animal as described in Methods. Data is expressed as mean ± S.E.M. ($n = 9-14$ mice for each group) for either male or female mice. ●●● $p < 0.001$ with respect to the SAL control group. ▲▲▲ $p < 0.001$ with respect to the MOR withdrawal group. ■■■ $p < 0.001$ with respect to the male MOR withdrawal group (ANOVA, Newman Keuls test).

syndrome in male mice but not in female mice. Second, blockade of both D_1 and D_2 receptors attenuates the expression of MOR withdrawal syndrome in both male and female mice.

The fact that we have performed our study with prepubertal mice (demonstrated by the vaginal smear) allows us to rule out hormonal cyclical changes characteristic of adult female mice which could greatly modify the endogenous concentrations of brain catecholamines (Hu et al., 2004). Although it is known that steroids influence sexual differentiation of brain morphology and neurobiology (Breedlove, 1992), taking into account our results, we could suggest that other factors than cyclical hormonal regulation may affect the dopaminergic brain system in mice.

Our results obtained in male mice are in general agreement with previous studies performed on male MOR withdrawn rodents (Pothos et al., 1991; Tokuyama et al., 1996; Acquas et al., 1991) which state that during the NAL-precipitated withdrawal, DA release is decreased in different brain areas. The present data clearly indicate a dimorphic response of the dopaminergic neurons during MOR withdrawal. This dimorphism was evidenciated by a marked decrease of the striatal and cortical dopaminergic activity in MOR withdrawn males while no changes in DA levels were observed in MOR withdrawn females. It is important to state that we measured the DA and DOPAC levels in brain homogenates, but a more precise characterization of these neurochemical sex differences might be obtained by microdialysis given that this technique enables the monitoring of extracellular molecules. A remarkable fact is that, even when it was not statistically significant, the cortical DA endogenous concentration decreased in MOR dependent females. This particular result is difficult to explain, considering that no other change occurs for DA or DOPAC levels in females. Moreover, there are no previous studies in female mice for contrasting this peculiar result. On the other hand, in the behavioral study, we have found a less sensitivity in female mice to develop the MOR withdrawal syndrome (comparing the global score between sex), which is in agreement with our previous results (Diaz et al., 2001). These observations could be explained at least partially, by the lack of changes observed in the endogenous monoamines brain levels of withdrawn female mice. In contrast, male mice were more prone to develop a full withdrawal syndrome, which could be related with significant changes of striatal and cortical DA and DOPAC levels.

Brain dopaminergic system has been widely implicated in the behavioral effects of opioids in males (Piepponen et al., 1996) but there is a lack of data about this interaction in females. Our behavioral results clearly show that both D_1 and D_2 receptors are involved in the expression of MOR withdrawal signs independently of the sex. Moreover, even when we showed the results as a global score, both antagonists equally attenuated the expression of the same signs in males as well as in females (data not shown). Previous studies have demonstrated different effects of dopaminergic antagonists on MOR-elicited responses. In that way, MOR-induced conditioned place preference in male mice was reversed by the administration of SCH 23390 and raclopride (Manzanedo et al., 2001). Likewise SCH 23390, but not raclopride, was able to counteract MOR withdrawal-induced aggression in male mice (Rodriguez-Arias et al., 1999). In an earlier study, other dopaminergic antagonists (SKF 83566 and sulpiride, D_1 and D_2 receptor antagonists, respectively) also produced inhibitory effects on NAL-induced withdrawal symptoms in mice (Samini et al., 2000). Our results are in general agreement with these previous observations performed in males and extend the current knowledges to female mice, suggesting that both D_1 and D_2 receptors are involved in the expression of MOR withdrawal syndrome in either sex.

A considerable amount of evidence indicates an interaction between opiates and the dopaminergic system. The nature of this interaction is complex because opiates produce effects that resemble both stimulation and inhibition of brain dopaminergic activity (El-Kadi and Sharif, 1998). The concept of autoregulation of dopaminergic neurons may partially explain this complexity: DA inhibits dopaminergic activity by acting on D_2 "autoreceptors" (Bunney, 1979; Roth, 1979). Therefore, MOR might influence DA-autoregulatory mechanisms, DA-stimulatory mechanisms or both (Cools et al., 1978; Iwamoto and Way, 1979). Taking into account the autoregulatory effect of D_2 receptors, the attenuation of MOR withdrawal signs that we have observed by administering raclopride could be due to an increased dopaminergic activity as a result of the D_2 receptor blockade. Given that during MOR withdrawal syndrome, DA release is dramatically decreased in different brain areas (Acquas and Di Chiara, 1992), the dopaminergic firing rate is abruptly and almost completely reduced (Diana et al., 1995) and DA levels are significantly decreased in some brain areas (Diaz et al., 2003), a possible increase in the

dopaminergic activity elicited by raclopride could be related to the attenuation of MOR withdrawal signs.

Our results also showed that the D_1 receptor antagonist SCH 23390 was able to decrease the global score obtained in males and females of the MOR withdrawal group. Although our results are in agreement with previous studies (Rodriguez-Arias et al., 1999; Manzanedo et al., 2001; Zarrindast et al., 2002) no explanation has been proposed for this effect. It is well known that opioids increase the firing of dopaminergic neurons in the ventral tegmental area (VTA) by presinaptic inhibition of GABA release (Simonato, 1996; Shoji et al., 1999). As stimulatory D_1 receptors are localized at GABAergic neurons both in the VTA (Cameron and Williams, 1993; Ranaldi and Wise, 2001) and striatum (Missale et al., 1998) one hypothesis to explain the effect of SCH 23390 on the MOR withdrawal could be that by blocking D1 receptors with SCH 23390, GABAergic inhibition could be abolished and thus, dopaminergic neurons could be disinhibited. Therefore, by increasing the dopaminergic activity, the expression of the MOR withdrawal signs may be attenuated.

In conclusion, DA and DOPAC levels were modified only in male mice by the MOR withdrawal syndrome. On the other hand, both D_1 and D_2 antagonists decreased the expression of MOR withdrawal signs in male and female mice. Taking into account the present findings, it would be of great interest to learn more about the mechanisms responsible for the sexual dimorphism in dopaminergic activity during MOR withdrawal. Additionally, the findings related to D_1 and D_2 receptors antagonists, add to a growing body of evidence indicating a critical role of dopaminergic system in the expression of the MOR withdrawal syndrome.

Acknowledgements

Our special thanks to Claudia García Bonelli for her excellent technical assistance. It is a pleasure to acknowledge Marina Cereseto, Irene Taravini and Leonel Joannas for their helpful assistance with mice administrations. We also extend our gratitude to the Parkinson group (ININFA) for its kind gift of SCH 23390.

This work was supported by Grant nos. JB32 and B090 from the University of Buenos Aires.

References

Acquas E, Di Chiara G. Depression of mesolimbic dopamine transmission and sensitization to morphine during opiate abstinence. J Neurochem 1992;58:1620–5.

Acquas E, Carboni E, Di Chiara G. Profound depression of mesolimbic dopamine release after morphine withdrawal in dependent rats. Eur J Pharmacol 1991;193:133–4.

Bassareo V, Tanda G, Di Chiara G. Increase of extracellular dopamine in the medial prefrontal cortex during spontaneous and naloxone-precipitated opiate-abstinence. Psychopharmacology (Berlin) 1995;122:202–5.

Breedlove SM. Sexual differentiation of the vertebrate nervous system. J Neurosci 1992;12:4133–42.

Bunney BS. The electrophysiological pharmacology of mid-brain dopaminergic systems. In: Horn AS, Koff JK, Westernik BHC, editors. The neurobiology of dopamine. New York: Academic Press; 1979. p. 417–52.

Cameron DL, Williams JT. Dopamine D1 receptors facilitate transmitter release. Nature 1993;366:344–7.

Candido J, Lutfy K, Billings B, Sierra V, Duttaroy A, Inturrisi CE, et al. Effect of adrenal and sex hormones on opioid analgesia and opioid receptor regulation. Pharmacol Biochem Behav 1992;42:685–92.

Cicero TJ, Nock B, Meyer ER. Sex-related differences in the morphine's antinociceptive activity: relationship to serum and brain morphine concentrations. J Pharmacol Exp Ther 1997;282:939–44.

Cools AR, Gieles LC, Janssen HJ, Megens AA. Morphine and its biphasic influence upon pharmacologically distinct dopaminergic systems within the feline caudate nucleus: a behavioural study. Eur J Pharmacol 1978; 48:67–85.

Craft RM, Stratmann JA, Bartok RE, Walpole TI, King SJ. Sex differences in development of morphine tolerance and dependence in rat. Psychopharmacology 1999;143:1–7.

Diana M, Pistis M, Muntoni A, Gessa G. Profound decrease of mesolimbic dopaminergic neuronal activity in morphine withdrawn rats. J Pharmacol Exp Ther 1995;272:781–5.

Diaz SL, Kemmling AK, Rubio MC, Balerio GN. Lack of sex-related differences in the prevention by baclofen of the morphine withdrawal syndrome in mice. Behav Pharmacol 2001;12:75–9.

Diaz SL, Kemmling AK, Balerio GN. Baclofen reestablishes striatal and cortical dopamine concentrations during naloxone-precipitated withdrawal. Neurochem Int 2003;42:293–8.

Diaz SL, Kemmling AK, Bonavita CD, Rubio MC, Balerio GN. Baclofen reestablishes μ-opioid receptor levels modified by morphine withdrawal syndrome in either sex. Synapse 2004;54:24–9.

El-Kadi AOS, Sharif SI. The role of dopamine in the expression of morphine withdrawal. Gen Pharmacol 1998;30:499–505.

Espejo EF, Serrano MI, Caille S, Stinus L. Behavioral expression of opiate withdrawal is altered after prefrontocortical dopamine depletion in rats: monoaminergic correlates. Neuropsychopharmacology 2001;25: 204–12.

Funada M, Shippenberg TS. Differential involvement of D_1 and D_2 dopamine receptors in the expression of morphine withdrawal signs in rats. Behav Pharmacol 1996;7:448–53.

Gianutsos G, Hynes M, Lal H. Enhancement of morphine-withdrawal and apomorphine-induced aggression by clonidine. Psychopharmacol Commun 1976;2:165–71.

Heikkila RE, Hess A, Duvoisin RC. Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. Science 1984;224:1451–3.

Hu M, Crombag HS, Robinson TE, Becker JB. Biological basis of sex differences in the propensity to self-administer cocaine. Neuropsychopharmacology 2004;29:81–5.

Iwamoto ET, Way EL. Opiate actions and catecholamines. Adv Biochem Psychopharmacol 1979;20:357–407.

Kantak K, Miczek K. Social, motor, and autonomic signs of morphine withdrawal: differential sensitivities to catecholaminergic drugs in mice. Psychopharmacology 1988;96:468–76.

Kest B, Palmese C, Hopkins E. A comparison of morphine analgesic tolerance in male and female mice. Brain Res 2000;879:17–22.

Koob GF, Bloom FE. Cellular and molecular mechanisms of drug dependence. Science 1988;242:715–23.

Maldonado R, Blendy JA, Tzavara E, Gass P, Roques BP, Hanoune J, et al. Reduction of morphine abstinence in mice with a mutation in the gene encoding CREB. Science 1996;273:657–9.

Manzanedo C, Aguilar MA, Rodriguez-Arias M, Miñarro J. Effects of dopamine antagonists with different receptor blockade profiles on morphine-induced place preference in male mice. Behav Brain Res 2001;121: 189–97.

Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. Physiol Rev 1998;78:189–225.

Piepponen TP, Katajamäki J, Kivastik T, Zharkovsky A, Ahtee L. Behavioural and neurochemical sensitization of morphine-withdrawn rats to quinpirole. Pharmacol Biochem Behav 1996;54:787–92.

Pothos E, Rada P, Mark GP, Hoebel BG. Dopamine microdialysis in the nucleus accumbens during acute and chronic morphine, naloxone-precipitated withdrawal and clonidine treatment. Brain Res 1991;566:348–50.

Ranaldi R, Wise RA. Blockade of D1 receptors in the ventral tegmental area decreases cocaine reward: possible role for dendritically released dopamine. J Neurosci 2001;21:5841–6.

Rodriguez-Arias M, Pinazo J, Minarro J, Stinus L. Effects of SCH 23390, raclopride, and haloperidol on morphine withdrawal-induced aggression in male mice. Pharmacol Biochem Behav 1999;64:123–30.

Roth RH. Dopamine autoreceptors: pharmacology, function and comparison with post-synaptic dopamine receptors. Commun Psychopharmacol 1979; 3:429–45.

Samini M, Fakhrian R, Mohagheghi M, Dehpour AR. Comparison of the effect of levodopa and bromocriptine on naloxone-precipitated morphine withdrawal symptoms in mice. Hum Psychopharmacol 2000;15:95–101.

Shoji Y, Delfs J, Williams JT. Presynaptic inhibition of GABAB-mediated synaptic potentials in the ventral tegmental area during morphine withdrawal. J Neurosci 1999;19:2347–55.

Simonato M. The neurochemistry of morphine addiction in the neocortex. Trends Pharmacol Sci 1996;17:410–5.

Stock HS, Caldarone B, Abrahamsen G, Mongeluzi D, Wilson MA, Rosellini RA. Sex differences in relation to conditioned fear-induced enhancement of morphine analgesia. Physiol Behav 2001;72:439–47.

Tidey JW, Miczek KA. Morphine withdrawal aggression: modification with D_1 and D_2 receptor agonists. Psychopharmacology 1992;108:177–84.

Tokuyama S, Wakabayashi H, Hoskins B, Ho IK. Naloxone-precipitated changes in biogenic amines and their metabolites in various brain regions of butorphanol-dependent rats. Pharmacol Biochem Behav 1996;54:461–8.

Zarrindast MR, Habibi M, Borzabadi S, Tabaei SF, Yahyavi SH, Rostamin P. The effects of dopamine receptor agents on naloxone-induced jumping behaviour in morphine-dependent mice. Eur J Pharmacol 2002;451:287–93.