Prodynorphin gene disruption increases the sensitivity to nicotine self-administration in mice

Lola Galeote, Fernando Berrendero, S. Andreea Bura, Andreas Zimmer and Rafael Maldonado

1 Laboratori de Neurofarmacologia, Departament de Ciències Experimentals i de la Salut, Universitat Pompeu Fabra, Barcelona, Spain
2 Institute of Molecular Psychiatry, Life & Brain Center, University of Bonn, Bonn, Germany

Abstract

The endogenous opioid system has been reported to participate in nicotine behavioural responses. The aim of the study was to determine the contribution of the endogenous peptides derived from prodynorphin in acute and chronic nicotine responses, mainly those related to its addictive properties. Locomotion and nociception were evaluated after acute nicotine administration in prodynorphin knockout mice. In addition, nicotine rewarding properties were investigated in the place-conditioning and the intravenous self-administration paradigms. The somatic signs of nicotine withdrawal were also analysed after the injection of the nicotinic antagonist mecamylamine in nicotine-dependent mice. The hypolocomotor and antinociceptive effects induced by acute nicotine administration were not modified in knockout (KO) animals. Nicotine also produced similar conditioned place preference in both genotypes. However, a shift to the left in the percentage of acquisition of intravenous nicotine self-administration was observed in prodynorphin KO mice. Indeed, a significant increase in the number of KO mice acquiring this operant behaviour was revealed when low doses of nicotine were used. Nicotine physical dependence was similar in wild-type and KO animals. These findings reveal a specific role of endogenous peptides derived from prodynorphin in nicotine self-administration, probably through the modulation of its aversive effects.

Received 8 July 2008; Reviewed 11 August 2008; Revised 22 August 2008; Accepted 22 August 2008;
First published online 21 October 2008

Key words: Conditioned-place preference, dynorphin, intravenous self-administration, nicotine, physical dependence.

Introduction

Nicotine is thought to be the most important compound in tobacco smoke that establishes and maintains tobacco dependence. Although the primary site of action of nicotine is the nicotinic acetylcholine receptor (nAChR) (Dani, 2001), different neurotransmitters seem to be involved in the behavioural effects of this drug. Recent reports indicate that some components of the endogenous opioid system play a role in the antinociceptive and addictive properties of nicotine.

Thus, nicotine antinociception was reduced in preproenkephalin knockout (KO) mice (Berrendero et al., 2005). This effect could be mediated through μ-opioid receptor (MOR) since nicotine antinociception was decreased in MOR KO mice (Berrendero et al., 2002) and was also attenuated by the opioid antagonist naloxone (Campbell et al., 2006; Kiguchi et al., 2008). MOR and the opioid peptides derived from preproenkephalin are also involved in nicotine rewarding responses and participate in the expression of nicotine physical dependence (Berrendero et al., 2002, 2005; Walters et al., 2005). In agreement, different clinical studies have suggested an involvement of opioids in the reinforcement of cigarette smoking, although the efficacy of the opioid antagonist naltrexone for smoking cessation is controversial (Schnoll and Lerman, 2006).

On the other hand, increasing evidence indicates that the dynorphin/k-opioid receptor (KOR) system
participants in the addictive properties of drugs of abuse (Shippenberg et al., 2007) by opposing their rewarding effects. Indeed, KOR agonists induce dysphoria in humans and aversive effects in experimental models (Hasebe et al., 2004). Accordingly, an attenuation of the dysphoric effects of natural and synthetic cannabinoids has been demonstrated in mice lacking the prodynorphin gene (Mendizabal et al., 2006; Zimmer et al., 2001). In addition, dynorphin A(1–17) blocked the increase in dopamine levels in the striatum and the conditioned place preference induced by cocaine in mice (Zhang et al., 2004). The overexpression of the transcription factor ΔFosB in the nucleus accumbens (NAc), partly through the repression of dynorphin expression, has been reported to increase the sensitivity to morphine rewarding effects (Zachariou et al., 2006). Recently, an increased dynorphin tone has been described during nicotine withdrawal in the mouse striatum (Isola et al., 2008), which could be responsible for the dysphoric state observed during nicotine abstinence.

In the present study, prodynorphin KO mice were used to evaluate the role of these endogenous opioid peptides in several pharmacological effects of nicotine related to its addictive properties.

Materials and methods

Animals

Male C57Bl/6J wild-type (WT) and prodynorphin KO mice were used in the present study. The generation of mice with a deletion of the dynorphin gene has been previously described (Zimmer et al., 2001). KO mice were backcrossed in a pure C57Bl/6J background for at least 10 generations. Mice (aged 10–16 wk) were housed five per cage in a temperature- (21 ± 0.5 °C) and humidity- (55 ± 10%) controlled room. For the self-administration study, mice were exposed to a reversed 12-h light/dark cycle (lights off 08:00 hours) and the conditioned place preference induced by cocaine in mice (Zhang et al., 2004). The overexpression of the transcription factor ΔFosB in the nucleus accumbens (NAc), partly through the repression of dynorphin expression, has been reported to increase the sensitivity to morphine rewarding effects (Zachariou et al., 2006). Recently, an increased dynorphin tone has been described during nicotine withdrawal in the mouse striatum (Isola et al., 2008), which could be responsible for the dysphoric state observed during nicotine abstinence.

In the present study, prodynorphin KO mice were used to evaluate the role of these endogenous opioid peptides in several pharmacological effects of nicotine related to its addictive properties.

Drugs

(−)-Nicotine hydrogen tartrate salt [(−)-1-methyl-2(3-pyridyl)pyrrolidine] and mecamylamine hydrochloride (Sigma, Madrid, Spain) were dissolved in physiological saline (0.9%) and administered subcutaneously in a volume of 10 ml/kg body weight. For the self-administration study, the pH of nicotine solution was adjusted to 7.4 with sodium hydroxide and was contingently administered by intravenous route. All nicotine doses were calculated as nicotine hydrogen tartrate salt. Ketamine hydrochloride (100 mg/kg) (Imalgène 1000; Rhône Mérieux, Lyon, France) and xylazine hydrochloride (20 mg/kg) (Sigma) were mixed and dissolved in ethanol (5%) and distilled water (95%). This anaesthetic mixture was administered intraperitoneally in an injection volume of 20 ml/kg body weight.

Acute pharmacological responses

The locomotor responses induced by acute administration of nicotine (1, 3 and 6 mg/kg s.c.) or saline were measured by using individual locomotor activity boxes (9 × 20 × 11 cm, Imetronic, Pessac, France), as previously described (Berrendero et al., 2002). Mice were placed in the locomotor cages 5 min after drug injection, and horizontal and vertical locomotor activity were recorded for 10 min in a low luminosity environment (20–25 lx).

The antinociceptive responses for each mouse were determined 15 and 16 min after nicotine (1, 3 and 6 mg/kg s.c.) or saline administration by using the tail-immersion and hot-plate tests respectively, as previously reported (Simonin et al., 1998). In the tail-immersion test, the water temperature was maintained at 50 ± 0.5 °C using a thermostatically regulated water circulating pump (Clifton, North Somerset, UK). The time taken to withdraw the tail was determined and a cut-off was set up at 5 s in order to prevent tissue damage. In the hot-plate test, the heated surface of the plate was kept at a temperature of 52 ± 0.1 °C (Columbus Instruments, Columbus, OH, USA). The nociceptive threshold evaluated was the jumping response. In absence of jumps, a 240 s cut-off was used to prevent tissue damage.

The data obtained were expressed as percentage of maximum possible effect (MPE) using the following equation:

\[
\% \text{ MPE} = \frac{\text{test latency} - \text{control latency}}{\text{cut-off time} - \text{control latency}} \times 100.
\]

Place-conditioning paradigm

The conditioned place preference to nicotine was performed using a non-biased procedure, as previously reported (Castañe et al., 2002). The apparatus
consisted of two main square conditioning compartments separated by a triangular central division. The movement and location of the mice were monitored by computerized monitoring software (Videotrack; View Point, Lyon, France). During the pre-conditioning phase, each mouse was placed in the middle of the central division and had free access to both compartments of the conditioning apparatus for 18 min, with the time spent in each compartment recorded. In the conditioning phase, mice were treated during 8 d with alternate injections of nicotine (0.5 mg/kg s.c.) or saline. Mice were confined in the corresponding compartment immediately after injection by using guillotine doors matching the walls for 20 min. The time in the central area was proportionally shared and added to the time value of each compartment, as previously reported (Maldonado et al., 1997). A score was calculated for each mouse as the difference between the post-conditioning and pre-conditioning time spent in the drug-paired compartment.

**Operant self-administration**

**Apparatus**

Self-administration was performed in operant chambers equipped with two nose-pokes. One nose-poke was selected as the active hole for delivering the drug and the other as the inactive hole. Infusions were delivered in a volume of 23.5 μl over 2 s. A stimulus light, located inside the active hole, was paired contingently with the delivery of the reinforcer.

**Surgery**

Mice were anaesthetized with a ketamine/xylazine mixture and then implanted with indwelling intravenous silastic catheters in the right jugular vein, as previously reported (Soria et al., 2005). The intravenous catheter patency was evaluated at the finish of the experiment by infusing 0.1 ml of thiopental (5 mg/ml) through the catheter. If prominent signs of anaesthesia were not apparent within 3 s of the infusion, the mouse was removed from the experiment.

**Drug self-administration procedure**

Sessions started 3 d after surgery. One-hour daily self-administration sessions were conducted 7 d/wk during 11 d under a fixed ratio 1 (FR1) schedule of reinforcement, followed by a 10-s time-out period, as previously reported (Soria et al., 2005). Nose-poking on the active hole resulted in a nicotine infusion (5.2, 10.6, 21.3, 42.7 and 85.5 μg/kg per infusion), while nose-poking on the inactive hole had no consequences. The number of infusions was limited to a maximum of 50 per session. Stable acquisition of self-administration behaviour was achieved when mice followed all the next criteria for at least three consecutive sessions: (1) less than 20% deviation from the mean of the total number of reinforcers (80% stability), (2) 75% of discrimination between holes, (3) a minimum of four infusions earned per session. Mice reaching these criteria were changed to a progressive ratio (PR) schedule of reinforcement on day 12, in which the response requirement to earn an infusion escalated according to the following series: 1–2–3–5–12–18–27–40–60–90–135–200–300–450–675–1000. Data from acquisition of nicotine self-administration were expressed as number of infusions and as area under the curve (AUC). AUC was calculated by using a standard trapezoid method,

\[
\text{AUC} = [0.5 \cdot (B_1 + B_2) \cdot h] + [0.5 \cdot (B_2 + B_3) \cdot h] + \ldots + [0.5 \cdot (B_n + B_{n+1}) \cdot h],
\]

where \(B_n\) were the infusions received for each mouse and \(h\) was the time (d) passed between the consecutive measurements (Gibaldi and Perrier, 1975).

**Nicotine dependence and withdrawal**

Nicotine dependence was induced by using Alzet osmotic minipumps (Model 2001; Alzet®, Cupertino, CA, USA), as previously reported (Castañe et al., 2002). These minipumps, implanted subcutaneously under brief ether anaesthesia, contained saline or nicotine solutions and delivered a constant subcutaneous flow in a rate of 1 μl/h. Pump delivery was verified by measuring the residual drug solution remaining in the pump reservoir at the time the pump was explanted. Mice received a mean dose of ~25 mg/kg.d of nicotine during 6 d. NICotine withdrawal was precipitated 6 d after minipump implantation by injection of the nicotinic receptor antagonist, mecamylamine (1 mg/kg s.c.). The somatic signs of withdrawal were evaluated immediately after mecamylamine injection during a period of 30 min, as previously reported (Castañe et al., 2002). A global withdrawal score was calculated for each animal by giving each individual sign a relative weight (Castañe et al., 2002).

**Statistical analysis**

Acute nicotine effects and global withdrawal scores were compared using a between-subjects two-way ANOVA (genotype and treatment as factors of variation), followed by one-way ANOVA and post-hoc
comparisons (Dunnett’s test) when required. For place-conditioning study, individual comparisons of time spent in the drug-paired compartment during pre-conditioning and test phases were made by paired two-tailed Student’s t test. To analyse the acquisition of nicotine self-administration at the different training doses, the number of infusions reached during the training period were compared using two-way ANOVA with repeated measures with day as within-subjects factor and genotype as between-subjects factor. AUC values were compared using two-way ANOVA with repeated measures with hole (active and inactive) as within-subjects factor and genotype as between-subjects factor, followed by one-way ANOVA when required. \( \chi^2 \) analysis was used to compare between genotypes the percentage of mice that acquired self-administration criteria at the different doses used. The breaking-point values obtained following the PR schedule and the dose–response curve values between genotypes were compared using the Mann–Whitney U test. Since data from dose–response curve and PR were not normally distributed, they were statistically analysed using non-parametric tests.

**Results**

**Nicotine effects on locomotor activity**

Nicotine (1, 3 and 6 mg/kg) decreased locomotor activity similarly in WT and prodynorphin KO mice (Figure 1a, b). Thus, two-way ANOVA calculated for horizontal locomotion showed a significant effect of treatment \( (F_{3,79} = 48.12, p < 0.0001) \), without genotype effect nor interaction between these two factors. Subsequent one-way ANOVA for treatment was also significant in WT \( (F_{3,40} = 17.65, p < 0.0001) \) and KO \( (F_{3,39} = 40.34, p < 0.0001) \) mice. Post-hoc analysis revealed a reduction of horizontal activity when nicotine was given at the dose of 1 mg/kg in KO mice \( (p < 0.01) \) and at 3 and 6 mg/kg in both genotypes \( (p < 0.01) \). Two-way ANOVA calculated for vertical locomotion showed a significant effect of treatment \( (F_{3,79} = 40.15, p < 0.0001) \), but no genotype effect nor interaction between both factors. One-way ANOVA for treatment was also significant in WT \( (F_{3,40} = 19.18, p < 0.0001) \) and KO \( (F_{3,39} = 33.68, p < 0.0001) \) mice. Post-hoc analysis revealed a significant reduction of vertical activity at doses of 1, 3 and 6 mg/kg \( (p < 0.01) \) in both genotypes.
Nicotine effects on antinociception

Nicotine-induced antinociception (1, 3 and 6 mg/kg) was similar in WT and prodynorphin KO mice (Figure 1c, d). In the hot-plate test, two-way ANOVA revealed a significant effect of treatment \((F_{1,38} = 42.58, p < 0.0001)\), but no effect of genotype nor interaction between both factors. Subsequent one-way ANOVA for treatment revealed significant effects in WT \((F_{3,46} = 19.32, p < 0.0001)\) and KO \((F_{3,38} = 26.64, p < 0.0001)\) mice. Post-hoc comparisons revealed a significant antinociceptive effect of nicotine at doses of 3 and 6 mg/kg \((p < 0.01)\) in both genotypes. In the tail-immersion test, two-way ANOVA revealed a significant effect of treatment \((F_{3,36} = 13.53, p < 0.0001)\) and genotype \((F_{1,36} = 11.36, p < 0.01)\), but no interaction between these two factors. Subsequent one-way ANOVA for WT \((F_{3,38} = 7.89, p < 0.05)\) and KO \((F_{3,38} = 8.48, p < 0.001)\) mice showed a significant effect of nicotine treatment. Post-hoc comparisons revealed similar nicotine antinociceptive responses at the dose of 6 mg/kg \((p < 0.01)\) in both genotypes.

Nicotine-induced conditioned place preference

One-way ANOVA revealed a similar time spent in the drug-paired compartment during the pre-conditioning phase in the different groups \((F_{3,30} = 0.35, \text{n.s.})\), ensuring the use of an unbiased procedure. Nicotine induced similar rewarding effects in the place-conditioning paradigm in WT and prodynorphin KO mice. Thus, WT and KO animals conditioned with a dose of 0.5 mg/kg nicotine spent significantly \((p < 0.01\) and \(p < 0.05\), respectively) more time in the drug-paired compartment during the testing phase than during the pre-conditioning phase (Figure 2).

Nicotine self-administration

WT and prodynorphin KO mice were trained to self-administer several doses of nicotine (5.2, 10.6, 21.3, 42.7 and 85.5 μg/kg per infusion) in order to evaluate the role of endogenous dynorphins in the reinforcing/aversive properties of the drug. WT animals were also tested for an additional higher dose of nicotine, 171 μg/kg per infusion and only 33.3% of mice reached the acquisition criteria at this dose (data not shown).

As shown in Figure 3a, significant differences between WT and prodynorphin KO mice were found in the percentage of mice that reached the acquisition criteria of nicotine self-administration. Thus, the percentage of acquisition at the lowest dose of nicotine (5.2 μg/kg per infusion,) was significantly decreased in WT mice compared to KO mice (16.6% vs. 50%) \((\chi^2 = 6.400, p < 0.05)\). However, at the dose of 85.5 μg/kg per infusion, the percentage of acquisition in WT mice was significantly enhanced compared to KO mice (55.5% and 22.2%, respectively) \((\chi^2 = 4.050, p < 0.05)\). These results revealed a shift to the left in the acquisition curve indicating that KO mice need lower doses of nicotine than WT animals to achieve similar percentages of acquisition. Accordingly, the analysis of the number of infusions during the acquisition period (Figure 4 and Table 1) and during the 3 d reaching the stability criteria (Figure 3b) showed similar results. Thus, a higher performance of operant nicotine self-administration was observed in mutant animals at the dose of 5.2 μg/kg per infusion, whereas WT mice showed a better performance at doses of 21.3 \((p < 0.05, \text{comparison of AUC and dose–response curve})\), 42.7 and 85.5 μg/kg per infusion of nicotine. In agreement, prodynorphin KO mice earned the highest number of infusions at low doses of nicotine (5.2–10.6 μg/kg per infusion), while the maximum responses in WT mice were observed at higher doses. The maximum effort to obtain a nicotine infusion evaluated under a PR schedule was similar in both genotypes when using doses of 5.2, 10.6, 21.3 and 85.5 μg/kg per infusion as revealed by the breaking-point values (Figure 3c). It was only when nicotine was self-administered at a dose of 42.7 μg/kg per infusion that a significantly higher breaking point was observed in WT mice compared to KO mice \((p < 0.05, \text{Mann–Whitney U test})\).
Nicotine physical dependence

During the behavioral observation performed before mecamylamine administration, no somatic signs of withdrawal were observed in any group of animals.

After mecamylamine injection, nicotine withdrawal syndrome was manifested by the presence of a variety of somatic signs in mice receiving chronic nicotine perfusion, as previously reported (Castan˜e et al., 2002).

The severity of nicotine withdrawal was not modified by genotype.  

**Table 1.** Two-way ANOVAs for acquisition of nicotine self-administration (Nic SA) and for area under curve (AUC) values at different training doses

<table>
<thead>
<tr>
<th>52 µg/kg per infusion</th>
<th>10.6 µg/kg per infusion</th>
<th>21.3 µg/kg per infusion</th>
<th>42.7 µg/kg per infusion</th>
<th>85.5 µg/kg per infusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Acquisition Nic SA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day (D)</td>
<td>F10,189 = 0.88 n.s.</td>
<td>F10,219 = 2.81 &lt; 0.01</td>
<td>F10,229 = 1.02 n.s.</td>
<td>F10,239 = 0.91 n.s.</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>F1,18 = 3.78 = 0.067</td>
<td>F1,21 = 0.14 n.s.</td>
<td>F1,22 = 4.90 &lt; 0.05</td>
<td>F1,24 = 1.80 n.s.</td>
</tr>
<tr>
<td>D × G</td>
<td>F10,189 = 0.41 n.s.</td>
<td>F10,219 = 0.32 n.s.</td>
<td>F10,229 = 0.88 n.s.</td>
<td>F10,239 = 0.72 n.s.</td>
</tr>
<tr>
<td><strong>AUC values</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hole (H)</td>
<td>F1,18 = 51.23 &lt; 0.01</td>
<td>F1,21 = 28.62 &lt; 0.01</td>
<td>F1,22 = 22.13 &lt; 0.01</td>
<td>F1,24 = 33.60 &lt; 0.01</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>F1,18 = 2.97 = 0.074</td>
<td>F1,21 = 0.06 n.s.</td>
<td>F1,22 = 9.39 &lt; 0.01</td>
<td>F1,24 = 2.05 n.s.</td>
</tr>
<tr>
<td>H × G</td>
<td>F1,18 = 3.60 = 0.074</td>
<td>F1,21 = 0.02 n.s.</td>
<td>F1,22 = 1.73 n.s.</td>
<td>F1,24 = 1.12 n.s.</td>
</tr>
</tbody>
</table>

Two-way ANOVA repeated measures with genotype as between-subjects factor and day or hole as within-subjects factor. (See Materials and methods section for details.)
by the deletion of the prodynorphin gene (Figure 5).
Thus, two-way ANOVA calculated for representative somatic signs of withdrawal such as body tremor, teeth chattering and ptosis indicated a significant effect of nicotine treatment ($F_{1,50}=20.60, p <0.0001$; $F_{1,50}=16.86, p <0.001$; $F_{1,50}=21.63, p <0.0001$, respectively), but no genotype effect ($F_{1,50}=0.27, n.s.$; $F_{1,50}=0.03, n.s.$; $F_{1,50}=0.00, n.s.$, respectively) nor interaction between treatment and genotype ($F_{1,50}=0.10, n.s.$; $F_{1,50}=0.00, n.s.; F_{1,50}=0.05, n.s.$, respectively). In agreement, two-way ANOVA for the global withdrawal score showed a significant effect of nicotine treatment ($F_{1,50}=17.64, p <0.001$), but not effect of genotype ($F_{1,50}=0.11, n.s.$) nor interaction between treatment and genotype ($F_{1,50}=0.11, n.s.$). Subsequent one-way ANOVA for treatment on individual signs and global withdrawal score values revealed a significant effect of nicotine in both WT and KO mice.

Figure 4. (a–c) Acquisition of nicotine self-administration in prodynorphin knockout (KO) mice [5.2 $\mu$g/kg per infusion ($n=8$); 10.6 $\mu$g/kg per infusion ($n=10$); 21.3 $\mu$g/kg per infusion ($n=9$); 42.7 $\mu$g/kg per infusion ($n=16$); 85.5 $\mu$g/kg per infusion ($n=9$)] and wild-type (WT) mice [5.2 $\mu$g/kg per infusion ($n=12$); 10.6 $\mu$g/kg per infusion ($n=13$); 21.3 $\mu$g/kg per infusion ($n=15$); 42.7 $\mu$g/kg per infusion ($n=10$); 85.5 $\mu$g/kg per infusion ($n=9$)]. (d) Area under the curve (AUC) values for the mean of infusions for 5.2, 10.6, 21.3, 42.7 and 85.5 $\mu$g/kg per infusion of nicotine (hydrogen tartrate salt) in both genotypes. Data are expressed as mean $\pm$ S.E.M. * $p <0.05$, ** $p <0.01$ for comparison between genotypes (one-way ANOVA). Data represent the mean of infusions in the active (●) and the inactive (○) holes in the 1-h sessions during 11 d of training.
Discussion

This study demonstrates for the first time the important role played by the opioid peptides derived from prodynorphin in the modulation of nicotine self-administration, probably through the mediation of its aversive effects. Indeed, a shift to the left of the dose–response curve was observed in the performance of nicotine self-administration in mice lacking the prodynorphin gene. However, nicotine-induced conditioned place preference, hypolocomotion and antinociception as well as nicotine physical dependence were not modified in KO animals.

Antinociception is a prototypical acute pharmacological effect of nicotine and nAChR agonists (Vincler, 2005). Previous reports have shown that enkephalins (Berrendero et al., 2005) and MOR (Berrendero et al., 2002; Campbell et al., 2006; Kiguchi et al., 2008) participate in the antinociceptive effects of nicotine. A recent report has also demonstrated that KOR contributes to spinal nicotine antinociception (Galeote et al., 2008). Nevertheless, deletion of the prodynorphin gene did not change the antinociceptive responses of nicotine in both the tail-immersion and hot-plate tests. In agreement with our results, it has been recently suggested that nicotine induces antinociception by promoting the release of Met-enkephalin, but not dynorphins, from opioidergic neurons in the spinal cord (Kiguchi et al., 2008). Similar divergences between the behavioural responses observed in KOR and those described in prodynorphin KO mice have been previously reported in the modulation of other responses such as morphine withdrawal (Simonin et al., 1998; Zimmer et al., 2001). A wider role of the prodynorphin products upon opioid receptors could explain this discrepancy. Thus, a large variety of opioid peptides that are not selective ligands of the KOR, are derived from the prodynorphin gene (Kakidani et al., 1982). In line with the results on antinociception, nicotine-induced hypolocomotion was unaffected by the dynorphin mutation, indicating that endogenous dynorphins do not play a relevant role in the acute locomotor effects of nicotine.

The conditioned place preference induced by nicotine (0.5 mg/kg) was similar in WT and prodynorphin KO animals indicating a lack of involvement of these endogenous opioid peptides in acute nicotine rewarding properties. This finding is in agreement with previous studies showing that place preference to ethanol, cocaine and morphine (Blednov et al., 2006; McLaughlin et al., 2003; Zimmer et al., 2001) was not modified in these mutant mice and suggests that the
endogenous peptides derived from prodynorphin are not required for the manifestation of the rewarding effects of drugs of abuse.

In contrast to the results obtained in the place-conditioning paradigm, a higher sensitivity to the reinforcing properties of nicotine was revealed in the operant self-administration experiments in prodynorphin KO mice, as revealed by the increased performance of these mutants when low doses of nicotine were used. Previous reports have shown that non-human primates (Le et al., 2007), rats (Corrigall and Coen, 1989), and mice (Fattore et al., 2002; Picciotto et al., 1998) acquire intravenous nicotine self-administration behaviour. However, some important differences between prior experiments performed in mice and the present study should be pointed out. In previous studies, mice were either trained first on cocaine (Picciotto et al., 1998) or food (Biklei-Gorzo et al., 2008) and had nicotine replaced afterwards, or the behaviour was evaluated in mice with restrained mobility (Fattore et al., 2002; Paterson et al., 2003). Interestingly, operant intravenous nicotine self-administration was evaluated in freely moving naive animals in the present study. This is an important advance since the reinforcing effects of nicotine are influenced by stress or the previous experience with food or other drugs (Thomsen and Caine, 2007), which complicated the interpretation of the preceding studies. Responding maintained by nicotine in this study was clearly dose-dependent in both control and mutant mice. Accordingly, nicotine exhibited an inverted U-shaped dose–effect curve in WT mice, with the doses of 5.2 and 171 µg/kg per infusion producing the lower percentages of mice achieving acquisition criteria (16.6 and 33.3%, respectively). The percentage of mice acquiring a stable self-administration behaviour was increased with intermediate doses (10.6–85.5 µg/kg per infusion). Interestingly, a facilitation of the reinforcing effects of nicotine was observed in prodynorphin KO mice since lower nicotine doses were required by these animals to acquire nicotine self-administration behaviour. Thus, lower doses of nicotine (5.2 and 10.6 µg/kg per infusion) were correlated with the highest percentage of acquisition (50% and 60%, respectively). A clear tendency to increase the number of infusions in the active hole was also found in mice lacking the prodynorphin gene at the dose of 5.2 µg/kg per infusion. Moreover, less intake of nicotine was required in KO animals to produce reinforcement at the dose of 21.3 µg/kg per infusion, which showed a similar percentage of acquisition in both genotypes.

Nicotine, as other addictive drugs, is known to produce both rewarding and aversive effects depending on the dose and individual vulnerability (Laviolette and van der Kooy, 2004). Thus, it has been suggested that people who become smokers are constitutionally more sensitive to the reinforcing consequences of nicotine (Pomerleau et al., 1998). The activation of the mesocorticolimbic dopaminergic system and subsequent increase of extracellular dopamine levels in the NAc (Berrendero et al., 2005; Di and Imperato, 1988; Pontieri et al., 1996; Zocchi et al., 2003) have been related to drug of abuse–induced rewarding effects. On the other hand, KOR and its endogenous dynorphin ligands are highly expressed in the NAc (Meshul and McGinty, 2000; Van Bockstaele et al., 1995) where they regulate the activity of mesolimbic dopamine neurons (Chefer et al., 2005). Extracellular dopamine levels in the NAc are elevated in mice lacking KOR (Chefer et al., 2005) whereas intrastriatal perfusion of dynorphin(1–17) decreases basal dopamine levels (Zhang et al., 2004). These results suggest that the KOR/dynorphin system maintains a tonic activity to inhibit dopamine neurotransmission in this region. Therefore, the absence of the inhibitory effect of dynorphin on dopamine release could explain the higher sensitivity to self-administer nicotine observed in mice lacking the prodynorphin gene. In accord, similar results have been obtained in these mutant mice with other drugs of abuse. Thus, a shift to the left in the dose intake curve for intravenous self-administration of the cannabinoid agonist WIN 55,212-2 (Mendizabal et al., 2006) and an abolishment of the conditioned place aversion induced by THC (Zimmer et al., 2001) have been reported in prodynorphin KO mice. This is also in agreement with previous studies showing that prior induction of dynorphin in the NAc attenuates the rewarding effects of cocaine (Carlezon, et al., 1998), whereas dynorphin repression in the same brain area increases morphine rewarding effects (Zachariou et al., 2006). In contrast, no differences in ethanol consumption were found between WT and male prodynorphin KO mice (Blednov et al., 2006).

Previous reports have demonstrated that endogenous enkephalins and MOR (Berrendero et al., 2005; Campbell et al., 2006) play a role in the expression of nicotine physical dependence. Nicotine withdrawal syndrome was not modified in the absence of the prodynorphin gene. A recent report has shown an increase of mRNA expression for prodynorphin in the NAc of nicotine-abstinent mice (Isola et al., 2008). Interestingly, the negative affective condition of nicotine abstinence is regulated by the endogenous opioid system since high doses of naloxone produced conditioned place aversion in chronically nicotine-treated rodents (Balerio et al., 2004; Ise et al., 2002; Watkins...
et al., 2000). Taken together, these results suggest that the dysphoric state associated to nicotine withdrawal, but not the somatic signs of dependence, could be regulated by opioid peptides derived from prodynorphin.

In summary, the present results show that nicotine can be self-administered in freely moving naive mice, and that endogenous peptides derived from prodynorphin modulate nicotine reinforcing properties in a negative manner. Therefore, the KOR/dynorphin system seems to be a common neurobiological substrate underlying the aversive properties of most drugs of abuse. The advancement in the characterization of the neurochemical substrate involved in nicotine addiction is of crucial relevance for the development of new rational therapeutic strategies for smoking cessation.

Acknowledgements

This work was supported by National Institute on Drug Abuse/National Institutes of Health (Extramural research project grant no. 1R01DA016768 to R.M., by the Instituto de Salud Carlos III grants, no. RD06/001/001 (RTA-RETICS) to R.M. and no. PI070559 (FIS) to F.B., by the Spanish Ministry of Science and Technology (Consolider-C, no. SAF2007-64062), and by the European Commission (IP no. LSHM-CT-2005SGR00131), by the Generalitat de Catalunya (no. 2005SGR00131), by the Spanish Ministry of Science and Technology (Consortium-C, no. SAF2007-64062), and by the European Commission (IP no. LSHM-CT-2004-05166, GENADDICT, and STREP no. LHS-M-CT-2006-037669, PHECOMP), to R.M. F.B. is a researcher supported by the Ramón y Cajal programme of the Spanish Ministry of Science and Technology.

Statement of Interest

None.

References


