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Effects of nandrolone on acute morphine responses, tolerance and dependence in mice

Evelyne Célérier[a], Maryam T. Yazdi[b], Anna Castañé[a], Sandy Ghozland[a], Fred Nyberg[b], Rafael Maldonado[a,*]

[a] *Laboratori de Neurofarmacologia, Facultat de Ciénces de la Salut i de la Vida, Universitat Pompeu Fabra, C/Doctor Aiguader 80, 08003 Barcelona, Spain*
[b] *Department of Pharmaceutical Biosciences, University of Uppsala, Box 591 Biomedicum, S751 24 Uppsala, Sweden*

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Abstract

Anabolic–androgenic steroid exposure has been proposed to present a risk factor for the misuse of other drugs of abuse. We now examined whether the exposure to the anabolic–androgenic steroid, nandrolone, would affect the acute morphine responses, tolerance and dependence in rodents. For this purpose, mice received nandrolone using pre-exposure (for 14 days before morphine experiments) or co-administration (1 h before each morphine injection) procedures. Nandrolone treatments increased the acute hypothermic effects of morphine without modifying its acute antinociceptive and locomotor effects. Nandrolone also attenuated the development of tolerance to morphine antinociception in the hot plate test, but did not affect tolerance to its hypothermic effects, nor the sensitisation to morphine locomotor responses. After nandrolone pre-exposure, we observed an attenuation of morphine-induced place preference and an increase in the somatic manifestations of naloxone-precipitated morphine withdrawal. These results indicate that anabolic–androgenic steroid consumption may induce adaptations in neurobiological systems implicated in the development of morphine dependence.

Keywords: Anabolic–androgenic steroid; Nandrolone; Hypothermia; Morphine tolerance; Reward; Dependence; (Mouse)

1. Introduction

Anabolic–androgenic steroids are synthetic derivates of testosterone widely used in the clinic as androgen replacement therapy and as chemotherapy for certain types of cancer (Kamel et al., 2001; Wilson and Griffin, 1980). However, there is also a wide nonmedical use of these steroids by athletes and others that has evoked considerable concern (Wilson, 1988). Indeed, during the last five decades, anabolic–androgenic steroids have been used at doses 10–100 times the therapeutic dose by many athletes and bodybuilders to enhance their physical performance, increase muscle mass and intensify training regimens (Kuhn, 2002; Lukas, 1993; Wilson, 1988; Yesalis and Bahrke, 1995). The original goal in making these drugs was to promote the anabolic effects of testosterone without its androgenic qualities. Although the androgenic effects were reduced, they were not eliminated, and this remains one of the main problems with anabolic steroids today (Marshall, 1988). Androgen action is linked to its ability to bind and activate specific androgen receptors (Falkenstein et al., 2000) throughout various regions of the brain, suggesting that anabolic–androgenic steroids may be involved in a wide variety of neural functions (Simerly et al., 1990). Effects of anabolic–androgenic steroids on physical and mental health have been widely reported (Pope and Katz, 1994). Thus, the development of psychological side-effects such as psychosis, increased irritability, hostility, aggression, loss of inhibition, lack of judgement, mood swings and increased self-esteem have been described in association with anabolic–androgenic steroid intake (Bahrke et al., 1996; Williamson and Young, 1992).

Interestingly, a concurrent abuse of anabolic–androgenic steroids has recently been reported among addicts not connected to sports (DuRant et al., 1993; Kindlundh et al., 1999; Lukas, 1993; Yesalis and Bahrke, 1995). Several studies have suggested the association between use of

* Corresponding author. Tel.: +34-93-542-28-45; fax: +34-93-542-28-02.
E-mail address: rafael.maldonado@cexs.upf.es (R. Maldonado).

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anabolic–androgenic steroids and consumption of alcohol, tobacco and psychotropic substances such as cannabis, opiates, amphetamine and ecstasy (DuRant et al., 1993; Kindlundh et al., 1999; Yesalis and Bahrke, 1995). Moreover, results of animal studies have indicated that anabolic–androgenic steroids evoke neurobiochemical alterations related to behavioural responses and reward in rats (Clark et al., 1996; Hallberg et al., 2000; Le Grevès et al., 1997; Schlussman et al., 2000; Thiblin et al, 1999), particularly by affecting the endogenous opioid and dopamine systems (Johansson et al., 1997, 2000a,b; Lukas, 1993; Menard et al., 1995). Based on these data, it has been proposed that anabolic–androgenic steroid may serve as "gateway" drugs to opioid dependence (Arvary and Pope, 2000).

The aim of this study was to use different behavioural models in mice in order to evaluate the effects of the anabolic–androgenic steroid, nandrolone, on acute morphine effects and on several behavioural responses induced by repeated morphine administration and related to its addictive properties. For this purpose, we first evaluated the influence of nandrolone on the changes in nociception, body temperature and locomotor activity induced by acute morphine administration. In a second step, we evaluated the development of tolerance to morphine antinociception, sensitisation to its locomotor effects and conditioned place preference after repeated morphine administration. We also investigated whether nandrolone exposure affects expression of the naloxone-precipitated morphine withdrawal syndrome.

2. Material and methods

2.1. Animals

Albino male CD-1 mice (CRIFFA, France) weighing 20–22 g after arrival were housed in cages of 10 and maintained at a controlled temperature (21 ± 1 °C) and humidity (55 ± 10%). The mice were given access to food and water ad libitum. Lighting was maintained at 12-h cycles (on at 8 a.m. and off at 8 p.m.). All the experiments were performed during the light phase of the dark/light cycle. The animals were habituated to the experimental room and handled for 1 week before the start of the experiments. All animal procedures met the guidelines of the National Institute of Health detailed in the "Guide for the Care and Use of Laboratory Animals", the European Communities directive 86/609/EEC regulating animal research and of the Local Ethical Committees.

2.2. Drugs

Morphine was provided by the Ministerio de Sanidad y Consumo (Spain). The anabolic–androgenic steroid, nandrolone decanoate, and naloxone were purchased from Sigma (Spain). Nandrolone was diluted in vehicle preparation (10% ethanol/10% cremophor EL/80% distilled water). All other compounds were dissolved in saline (0.9%). All the compounds were administered in a volume of 10 ml/kg.

2.3. Experimental procedure

Four series of experiments were performed in order to evaluate the effects of nandrolone treatment on acute and chronic morphine effects. Two different protocols (co-administration or pre-exposure) were used for nandrolone administrations in an attempt to represent conditions similar to its use by drug addicts (drug intake concomitant to nandrolone use or drug intake with a past of long-term consumption of nandrolone). In the first protocol (pre-exposure), nandrolone (15 mg/kg, i.m.) was administered chronically once daily for 14 days before starting morphine treatment. In the second protocol (co-administration), nandrolone (15 mg/kg, i.m.) was administered 1 h before each morphine injection. In the pre-exposure protocol, the intramuscular injections were given in the left and right hind legs, alternatively. A supratherapeutic dose of nandrolone was used (i) because this mimics the dose self-administered by heavy nandrolone abusers (Williamson and Young, 1992) and (ii) because it has previously been shown to induce biochemical changes in the endogenous opioid and dopamine systems in rodents (see Johansson et al., 1997).

All the experiments included four groups, receiving vehicle and morphine, nandrolone and morphine, vehicle and saline, or nandrolone and saline.

2.3.1. Acute pharmacological effects of morphine

The acute effects induced by different doses of morphine (1, 3 or 9 mg/kg, s.c.) on nociception, locomotion and body temperature were evaluated in each experimental group.

Two nociceptive tests were used, the tail immersion and the hot plate. In the tail immersion test, mice were gently placed in a restrainer cylinder. The nociceptive threshold was assessed as described previously (Janssen et al., 1963), by measuring the time to withdraw the tail immersed in a thermostated water bath (50 ± 0.1 °C) (Clifton, Scientific Instruments, England), with a cutoff latency of 15 s to prevent tissue damage. Nociceptive responses were also measured using a hot plate analgesia meter (Columbus, OH, USA). A glass cylinder (19 cm high, 19 cm diameter) was used to keep the mice on the heated surface of the plate, which was maintained at a temperature of 52 ± 0.1 °C. Two nociceptive thresholds, licking of the paws and jumping, were evaluated. The cutoff time was 30 and 240 s, respectively.

Locomotor responses were evaluated using locomotor activity boxes (9 × 20 × 11 cm; Imetronic, Lyon, France). The boxes contained a line of photocells 2 cm above the floor to measure horizontal movements and another line located 6 cm above the floor to measure vertical activity (rearing). On the experimental day, the mice were individually placed in the boxes and the ambulatory, horizontal

(ambulatory movements plus small movements) and vertical activities were recorded for 10 min in a low luminosity environment (5–15 lx).

Rectal temperature was measured in each mouse using an electronic thermocouple flexible probe (Panlab, Barcelona, Spain). The probe was placed 3 cm into the rectum of the mice for 20 s before the temperature was recorded.

In order to habituate the animals to the test environment and to obtain a stable baseline, tail immersion response and locomotor activity were measured for 2 days before the experiment. On the experimental day, locomotor activity was evaluated 10 min after morphine injection (1, 3 or 9 mg/kg, s.c.) during 10 min. Then (i.e. 20 min after morphine), the tail immersion test was performed, immediately followed by the hot plate test. Rectal temperature was measured just before and 40 min after the morphine injection.

2.3.2. Tolerance to the morphine antinociceptive effects and sensitisation to its locomotor responses

As in the previous experiment, the basal tail immersion response and locomotor activity were evaluated for 2 days before the beginning of the chronic morphine treatment on day 1. Morphine (12 mg/kg, s.c.) was administered chronically twice a day (12-h interval) for 14 days (days 1–14). Locomotor activity was recorded 10 min after the morning morphine injection on days 1, 2, 4, 6, 8, 10 and 12. Changes in nociceptive threshold were assessed 20 min after morning injection of 3 and 9 mg/kg (s.c.) of morphine using the tail immersion test (days 0, 7 and 14) and the hot plate test (day 14). The morning of day 7, animals received only this morphine injection. Morphine-induced changes in body temperature were also evaluated on day 14. Data for antinociceptive tolerance were expressed as the percentage of maximal possible effect (%MPE). The calculation of the %MPE was performed using the following formula: (test latency − control mean latency)/(cutoff − control mean latency) × 100.

2.3.3. Rewarding effects of morphine

The rewarding effects of morphine were evaluated using the conditioned place preference paradigm, as previously described (Maldonado et al., 1997). The place preference apparatus consisted of two different cubic compartments (15 × 15 × 15 cm) separated by a triangular central neutral area (15 cm per side). The place preference conditioning schedule consisted of three phases. During the preconditioning phase, the mouse was placed in the middle of the neutral area and allowed to explore both compartments, and the time spent in each compartment was measured for 18 min. After the session, animals were randomised for pairing to drug or vehicle administration and for assignment to a compartment. Care was taken to ensure that treatments were counterbalanced as closely as possible between compartments. During the conditioning phase, the animals were treated for 6 consecutive days with an injection of morphine (5 mg/kg, s.c.) or saline. Doors matching the walls of the compartment allowed confinement of the mice for 20 min immediately after morphine or saline injections. Mice received morphine on days 1, 3 and 5 and vehicle on days 2, 4 and 6. Control animals received vehicle every day. Finally, the test phase was conducted 24 h after the last conditioning session exactly as was the preconditioning phase, i.e. free access to both compartments and the time spent in each compartment measured for 18 min. A score was calculated for each mouse as the difference between the post-conditioning and the pre-conditioning time spent in the drug-paired compartment.

2.3.4. Naloxone-precipitated morphine withdrawal

The naloxone-precipitated withdrawal syndrome in morphine-dependent mice was evaluated as previously described (Maldonado et al., 1997). Morphine was injected i.p. twice a day (9 a.m. and 9 p.m.) for 6 days. The morphine dose was progressively increased as follows: 1st day, 20 mg/kg; 2nd day, 40 mg/kg; 3rd day, 60 mg/kg; 4th day, 80 mg/kg; 5th day, 100 mg/kg; 6th day (only morning injection), 100 mg/kg. Control mice were treated with saline under the same conditions. Withdrawal was precipitated in each animal by injecting naloxone (1 mg/kg, s.c.) 2 h after the last morphine administration. The animals were placed individually into test chambers to evaluate the behavioural signs of withdrawal. The chambers consisted of transparent round plastic boxes (30 cm in diameter and 50 cm in height) with a white floor. Behaviour was observed in two sessions: the first session was during the 15 min preceding naloxone injection and the second session was for 30 min immediately after this injection. The wet dog shakes, jumping, paw tremor and sniffing were counted. Teeth chattering, piloerection and ptosis were evaluated over 5-min periods, 1 point being given for the presence of each sign during each period. The periods with a sign were then counted (maximum score: 6). Body weight was determined before and 30 min after naloxone injection. Taking into account all the individual signs, a global withdrawal score was calculated for each animal by using a range of possible scores from 0 to 100, as previously reported (Maldonado et al., 1992a).

2.4. Statistical analysis

For the analysis of the data obtained for acute morphine pharmacological responses, a one-way analysis of variance (ANOVA) was first used to determine the doses of morphine that produced significant effects. A two-way ANOVA was then performed to evaluate the influence of the nandrolone treatment in the groups of mice where significant morphine effects were observed. Consecutive post hoc comparisons, using Dunnett's test, were performed when appropriate. The development of tolerance to morphine antinociception in the hot plate test and the effects on the naloxone-precipitated morphine withdrawal syndrome were analysed by using a two-way ANOVA with nandrolone/vehicle and morphine/saline treatments as factors of variation. One-way ANOVAs

were used to reveal main morphine or nandrolone effect. Data from the study of development of tolerance to morphine antinociception in the tail immersion test and sensitisation to locomotor activities were analysed using a three-way ANOVA with one within-subjects (time), and two between-subjects (nandrolone/vehicle and morphine/saline treatments) factors of variation. When required, the three-way ANOVA was followed by two-way and one-way ANOVAs, as well as by post hoc comparisons (Dunnett's test). In conditioning place preference experiments, individual comparisons of time spent in the drug-paired compartment during preconditioning and test phases were made in each experimental group by using the paired two-tailed Student's *t*-test. Additionally, one-way ANOVA was used to compare the score values, followed by consecutive post hoc comparisons (Dunnett's test). The statistical significance criterion was $P < 0.05$.

3. Results

3.1. Effects of nandrolone treatments on acute morphine responses

The influence of nandrolone pre-exposure and co-administration on the acute pharmacological effects of morphine (1, 3 and 9 mg/kg, s.c.) was evaluated by measuring the changes induced in nociception, locomotor activity and body temperature. Control experiments showed that nandrolone had no intrinsic effect when injected in combination with saline on any of the parameters studied (data not shown).

Chronic nandrolone pre-exposure for 14 days did not influence acute morphine effects on nociception as evaluated in tail immersion (Fig. 1A) and hot plate (Fig. 1B and C) tests. In the tail immersion test, significant antinociceptive effects of 3 and 9 mg/kg of morphine were revealed in vehicle-pre-exposed mice (one-way ANOVA, $F(3,39) = 18.14$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(2,59) = 25.46$, $P < 0.001$) but no nandrolone effect ($F(1,59) = 1.56$, nonsignificant, NS) and no morphine/nandrolone interaction ($F(2,59) = 1.18$, NS). For the licking threshold in the hot plate test, significant antinociceptive effects of 3 and 9 mg/kg of morphine were revealed in vehicle pre-exposed mice (one-way ANOVA, $F(3,38) = 14.25$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(2,59) = 51.70$, $P < 0.001$), no nandrolone effect ($F(1,59) = 0.04$, NS) and a significant morphine/nandrolone interaction ($F(2,59) = 4.24$, $P < 0.05$), but subsequent one-way ANOVA revealed no significant differences between the different vehicle and nandrolone pre-exposed groups (morphine 3 mg/kg: $F(1,19) = 3.06$, NS; morphine 9 mg/kg: $F(1,18) = 3.83$, NS). For the jumping threshold in the hot plate test, significant antinociceptive effects of 3 and 9 mg/kg of morphine were revealed in vehicle-co-treated mice (one-way ANOVA, $F(3,37) = 26.66$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(2,51) = 154.21$, $P < 0.001$) but no nandrolone effect ($F(1,51) = 0.01$, NS) and no morphine/nandrolone interaction ($F(2,51) = 0.76$, NS). Similar morphine dose-dependent antinociceptive responses were obtained in both vehicle and nandrolone-co-treated mice as shown by the nociceptive threshold in the tail immersion (Fig. 1E) and hot plate (Fig. 1F and G) tests. In the tail immersion test, significant antinociceptive effects of 3 and 9 mg/kg of morphine were revealed in vehicle-co-treated mice (one-way ANOVA, $F(3,68) = 24.59$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(2,96) = 42.57$, $P < 0.001$) but no nandrolone effect ($F(1,96) = 2.56$,

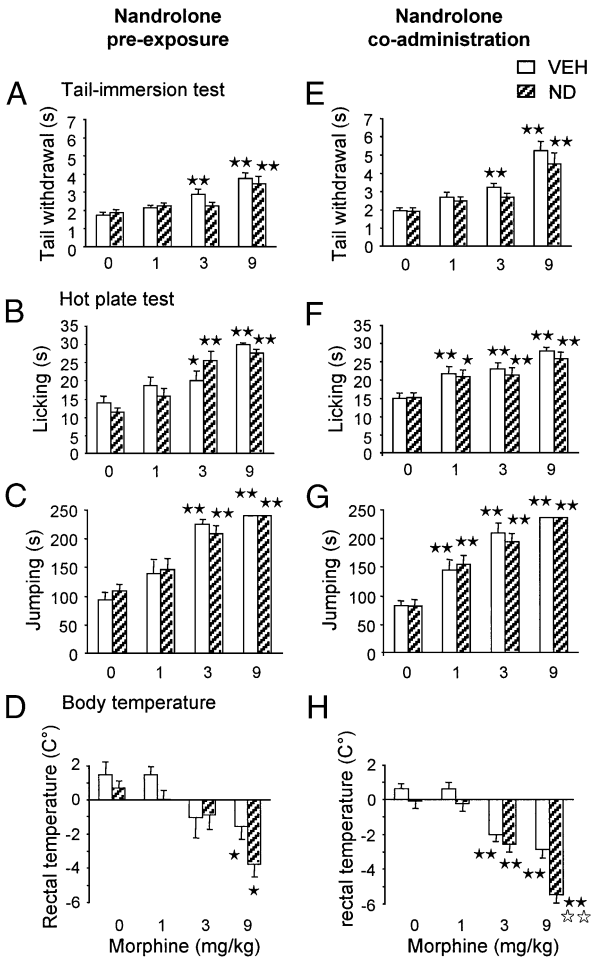

Fig. 1. Acute effects of morphine (1, 3 and 9 mg/kg, s.c.) on nociception (A–C and E–G) and body temperature (D and H) in mice pre-exposed to or co-treated with nandrolone (ND) or vehicle (VEH). Two behavioural tests were used to evaluate changes in nociception: the tail immersion (A and E) and hot plate (licking threshold: B and C; jumping threshold: F and G) tests. Antinociceptive effects were evaluated 20 min after morphine injection. The rectal temperature was measured just before and 40 min after morphine injection. Number of mice per group in the nandrolone co-treatment experiments = 19–20. Number of mice per group in the nandrolone pre-exposure experiments = 9–10. Data are expressed as means ± S.E.M. $^{\star}P < 0.05$, $^{\star\star}P < 0.01$ (Dunnett's test, comparison with the respective saline control group) and $^{\star\star}P < 0.01$ (one-way ANOVA, VEH- vs. ND-treated mice).

NS) and no morphine/nandrolone interaction ($F(2,96) = 0.65$, NS). In the licking threshold in the hot plate test, significant antinociceptive effects of 1, 3 and 9 mg/kg of morphine were revealed in vehicle co-treated mice (one-way ANOVA, $F(3,68) = 15.90$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(3,128) = 22.28$, $P < 0.001$) but no nandrolone effect ($F(1,128) = 1.38$, NS) and no morphine/nandrolone interaction ($F(3,128) = 0.17$, NS). For the jumping threshold in the hot plate test, significant antinociceptive effects of 1, 3 and 9 mg/kg of morphine were revealed in vehicle-co-treated mice (one-way ANOVA, $F(3,69) = 44.95$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(3,136) = 79.71$, $P < 0.001$) but no nandrolone effect ($F(1,136) = 0.07$, NS) and no morphine/nandrolone interaction ($F(3,136) = 0.43$, NS).

Nandrolone pre-exposure and co-administration had different effects on the hypothermic responses to morphine (Fig. 1D and H). In mice chronically pretreated with vehicle (Fig. 1D), significant hypothermia was found after treatment with the dose of 9 mg/kg of morphine (one-way ANOVA, $F(3,38) = 4.10$, $P < 0.05$). Two-way ANOVA revealed a significant hypothermic effect of 9 mg/kg of morphine in nandrolone pre-exposed mice, but no significant difference was found from the vehicle pre-exposed control group (morphine effect: $F(1,38) = 35.57$, $P < 0.001$; nandrolone effect: $F(1,38) = 5.59$, $P < 0.05$; and no morphine/nandrolone interaction: $F(2,38) = 1.29$, NS).

In the nandrolone co-administration groups, significant hypothermia was revealed after 3 and 9 mg/kg of morphine in vehicle co-treated mice (Fig. 1H, one-way ANOVA, $F(3,48) = 20.41$, $P < 0.001$). Two-way ANOVA revealed a significant morphine effect ($F(2,73) = 53.14$, $P < 0.001$), a nandrolone effect ($F(1,73) = 12.78$, $P < 0.01$) and a morphine/nandrolone interaction ($F(2,73) = 7.77$, $P < 0.05$). Subsequent one-way ANOVA revealed a significantly increased hypothermic effect of 9 mg/kg of morphine in nandrolone-co-treated mice as compared to the vehicle-co-treated group ($F(1,23) = 11.92$, $P < 0.01$).

Nandrolone pre-exposure and co-administration did not produce major changes in the effects of morphine on ambulatory, horizontal and vertical activities (data not shown). In nandrolone pre-exposed mice, two-way ANOVA revealed a significant morphine effect on ambulatory ($F(3,79) = 5.92$, $P < 0.01$), horizontal ($F(3,76) = 3.77$, $P < 0.05$) and vertical ($F(2,56) = 22.02$, $P < 0.001$) activities. However, no significant effect of nandrolone (ambulatory: $F(1,79) = 0.26$, NS; horizontal: $F(1,76) = 1.14$, NS; vertical: $F(1,56) = 1.79$, NS) and no morphine/nandrolone interaction (ambulatory: $F(3,79) = 0.35$, NS; horizontal: $F(3,76) = 1.15$, NS; vertical: $F(2,56) = 1.50$, NS) was observed in any case. In nandrolone co-treated mice, one-way ANOVA ($F(3,69) = 10.67$, $P < 0.01$) and Dunnett's test post hoc comparisons indicated that 9 mg/kg of morphine significantly ($P < 0.05$) increased ambulatory activity in vehicle co-treated mice. Two-way ANOVA revealed a significant morphine effect ($F(1,70) = 8.06$, $P < 0.01$) but no nandrolone effect ($F(1,66) = 2.87$, NS) and no morphine/nandrolone interaction ($F(1,66) = 1.12$, NS). One-way ANOVA ($F(3,68) = 3.55$, $P < 0.05$) and Dunnett's test post hoc comparisons revealed an inhibitory effect of 3 mg/kg of morphine ($P < 0.05$) on horizontal activity of vehicle-co-treated mice. Two-way ANOVA revealed a significant morphine effect ($F(1,66) = 11.46$, $P < 0.01$) but no nandrolone effect ($F(1,66) = 0.02$, NS) and no morphine/nandrolone interaction ($F(1,66) = 0.31$, NS). One-way ANOVA ($F(3,64) = 15.88$, $P < 0.001$) and Dunnett's test post hoc comparisons indicated that 1, 3 and 9 mg/kg of morphine significantly ($P < 0.001$) decreased vertical activity in vehicle co-treated mice. Two-way ANOVA revealed a significant morphine effect ($F(3,129) = 22.23$, $P < 0.001$) but no nandrolone effect ($F(1,129) = 0.48$, NS) and no morphine/nandrolone interaction ($F(3,129) = 1.10$, NS).

3.2. Effects of nandrolone treatments on the development of tolerance to morphine antinociception

Nandrolone pre-exposure and co-administration did not produce any change in the development of tolerance to antinociception produced by chronic morphine administration (12 mg/kg, s.c., twice a day for 14 days) as evaluated by the tail immersion test (Fig. 2). The changes in nociceptive threshold were evaluated on days 0, 7 and 14 of treatment. Nandrolone treatments did not modify the tail-withdrawal latencies in saline-treated control mice (Fig. 2). In morphine chronically treated mice, tolerance to the antinociceptive effects of morphine developed similarly in both vehicle and nandrolone-pre-exposed mice (Fig. 2A–C). Three-way ANOVA revealed a morphine effect ($F(2,79) = 43.61$, $P < 0.001$), a time effect ($F(2,158) = 41.89$, $P < 0.001$) and a morphine/time interaction ($F(4,158) = 14.95$, $P < 0.001$), but no nandrolone effect ($F(1,79) = 1.90$, NS) and no morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(2,79) = 1.70$, $F(2,158) = 0.53$ and $F(4,158) = 0.96$, respectively, NS). Similar results were obtained in morphine mice co-treated with nandrolone (Fig. 2D–F). Three-way ANOVA revealed a morphine effect ($F(2,87) = 39.50$, $P < 0.001$), a time effect ($F(2,174) = 12.15$, $P < 0.001$) and a morphine/time interaction ($F(4,174) = 6.59$, $P < 0.001$), but no nandrolone effect ($F(1,87) = 0.23$, NS) and no morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(2,87) = 0.29$, $F(2,174) = 1.26$ and $F(4,174) = 0.13$, respectively, NS).

On the contrary, a nandrolone effect was found on the development of tolerance to morphine antinociception as evaluated by the hot plate test on day 14 of opioid treatment (jumping threshold; Fig. 3). Tolerance to the antinociceptive effects of 9 mg/kg of morphine was observed in vehicle-pre-exposed mice (-47%), but not in nandrolone-pre-exposed animals (-6%). Two-way ANOVA revealed a significant time effect ($F(1,47) = 12.75$, $P < 0.01$), a nandrolone effect ($F(1,47) = 17.34$, $P < 0.01$) and a nandrolone/time interaction ($F(1,47) = 7.34$, $P < 0.01$). Significant differences be-

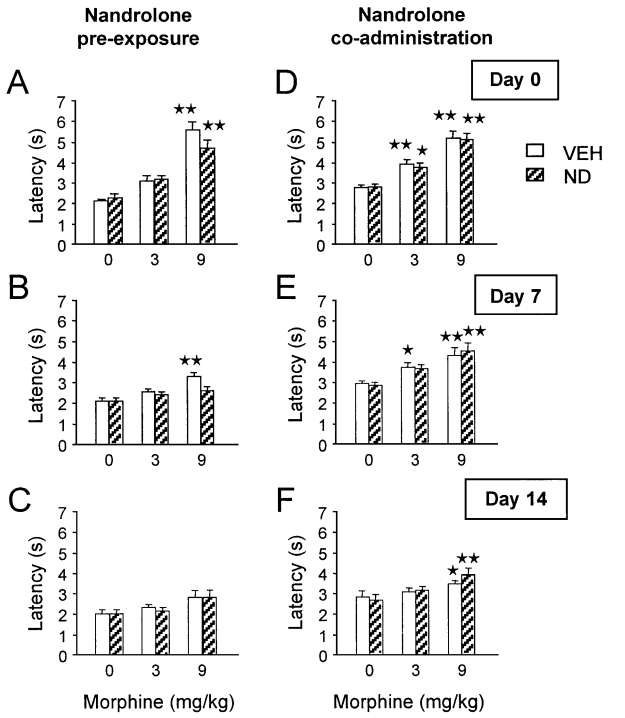

Fig. 2. Tolerance to morphine-induced antinociception in the tail immersion test in mice pre-exposed to (A–C) or co-treated with (D–F) nandrolone (ND) or vehicle (VEH). Mice received repeated morphine administration (12 mg/kg, s.c., twice a day for 12 days). Nociceptive threshold was evaluated 20 min after the injection of 3 and 9 mg/kg (s.c.) of morphine on the morning of days 0, 7 and 14. Number of mice per group in the nandrolone co-treatment experiments = 15. Number of mice per group in the nandrolone pre-exposure experiments = 10–16. Data are expressed as means ± S.E.M. $^{\star}P<0.05$, $^{\star\star}P<0.01$ (Dunnett's test, comparison with the respective saline control group) and $^{\star\star}P<0.01$ (one-way ANOVA, VEH- vs. ND-treated mice).

tween nandrolone- and vehicle-co-treated mice appeared on day 14 ($F(1,25)=6.83$, $P<0.01$). This effect of nandrolone pre-exposure was not observed with the lower dose of morphine (3 mg/kg). In this case, tolerance to antinociception developed to a similar degree in vehicle- (−86%) and nandrolone-pre-exposed mice (−74%). Two-way ANOVA revealed a significant time effect ($F(1,46)=62.59$, $P<0.001$), no nandrolone effect ($F(1,46)=0.01$, NS) and no nandrolone/time interaction ($F(1,46)=3.05$, NS).

In the co-administration experiments, nandrolone attenuated the development of tolerance to morphine antinociception for both 3 and 9 mg/kg doses of morphine (Fig. 3C and D). Tolerance to the antinociceptive effects of 3 mg/kg of morphine developed to a lower degree in nandrolone-co-treated mice (−74%) as compared to the vehicle-treated group (−100%). Two-way ANOVA revealed a significant time effect ($F(1,60)=79.39$, $P<0.001$), no nandrolone effect ($F(1,60)=0.78$, NS) and a significant nandrolone/time interaction ($F(1,60)=4,46$, $P<0.05$). Significant differences between the nandrolone- and vehicle-co-treated groups appeared on day 14 ($F(1,30)=7.17$, $P<0.05$). Moreover, tolerance to the antinociceptive effects of 9 mg/kg of morphine developed in vehicle-co-treated mice (−42%), but not in nandrolone-treated animals (−4%). Two-way ANOVA revealed a significant time effect ($F(1,61)=18.08$, $P<0.001$), a nandrolone effect ($F(1,61)=10.75$, $P<0.01$) and a nandrolone/time interaction ($F(1,61)=10.75$, $P<0.01$). Significant differences between nandrolone and vehicle co-treated mice also appeared on day 14 ($F(1,31)=10.42$, $P<0.01$).

3.3. Effects of nandrolone treatments on the development of tolerance to morphine hypothermia

The influence of nandrolone on the development of tolerance to morphine-induced hypothermia was also evaluated (data not shown).

Nandrolone pre-exposure did not modify the development of tolerance to the hypothermic effects of 3 mg/kg of morphine (two-way ANOVA: time effect, $F(1,47)=48.00$, $P<0.01$; nandrolone effect, $F(1,47)=0.04$, NS; nandrolone/time interaction, $F(1,47)=0.14$, NS). A similar development of tolerance to the hypothermic effects of 9 mg/kg morphine was also observed in both groups (two-way ANOVA: time effect, $F(1,48)=30.54$, $P<0.001$; nandrolone effect, $F(1,48)=8.60$, $P<0.01$; nandrolone/time interaction, $F(1,48)=1.92$, NS).

The development of tolerance to the hypothermic effects of 3 mg/kg of morphine on day 14 was similar in both vehicle- and nandrolone-co-treated mice (two-way ANOVA: time effect, $F(1,50)=23.11$, $P<0.001$; nandrolone effect, $F(1,50)=0.57$, NS; nandrolone/time interaction, $F(1,50)=0.65$,

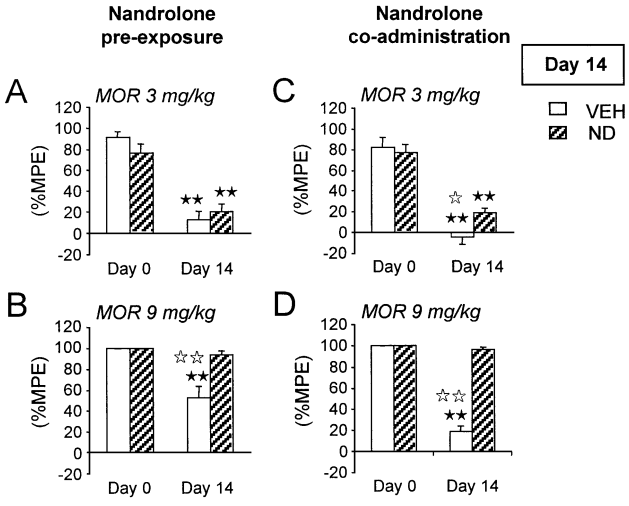

Fig. 3. Tolerance to morphine-induced antinociception in the hot plate (jumping) test in mice (A and B) pre-exposed to or co-treated with (C and D) nandrolone (ND) or vehicle (VEH). Mice received repeated morphine administration (MOR, 12 mg/kg, s.c., twice a day for 14 days). Jumping threshold was evaluated 20 min after the injection of 3 and 9 mg/kg (s.c.) of morphine on the morning of day 14. Number of mice per group in the nandrolone pre-exposure experiments = 10–16. Number of mice per group in the nandrolone co-treatment experiments = 15. Data are expressed as means ± S.E.M. $^{\star\star}P<0.01$ (one-way ANOVA, Day 0 vs. Day 14); $^{\star}P<0.05$, $^{\star\star}P<0.01$ (one-way ANOVA, VEH- vs. ND-treated mice).

NS). Tolerance to the hypothermic effects of 9 mg/kg morphine also developed in a similar way in both vehicle- and nandrolone-co-treated mice (two-way ANOVA: time effect, $F(1,52)=43.19$, $P<0.001$; nandrolone co-treatment effect, $F(1,52)=0.89$, NS; nandrolone/time interaction, $F(1,52)=2.95$, NS).

3.4. Effects of nandrolone treatments on morphine-induced locomotor sensitisation

Nandrolone pre-exposure did not modify the ability of morphine to elicit sensitisation to ambulatory movements (Fig. 4A). Three-way ANOVA revealed a significant morphine effect ($F(1,81)=53.33$, $P<0.001$), a time effect ($F(7,567)=14.13$, $P<0.001$) and a morphine/time interaction ($F(7,567)=16.22$, $P<0.001$), but no nandrolone effect ($F(1,81)=0.04$, NS) and no morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(1,81)=0.07$, $F(7,567)=0.29$ and $F(7,567)=0.51$, respectively, NS). Nandrolone pre-exposure also did not modify morphine-induced sensitisation of horizontal activity (Fig. 4B). Three-way ANOVA revealed a significant morphine effect ($F(1,81)=30.37$, $P<0.001$), a time effect ($F(7,567)=12.36$, $P<0.001$) and a morphine/time interaction ($F(7,567)=12.08$, $P<0.001$), but no nandrolone effect ($F(1,81)=0.58$, NS) and no morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(1,81)=0.01$, $F(7,567)=0.74$ and $F(7,567)=0.48$, respectively, NS). We also found no influence of nandrolone pre-exposure on the morphine-induced decrease in vertical locomotion (data not shown). Three-way ANOVA revealed a significant morphine effect ($F(1,81)=8.41$, $P<0.001$), a time effect ($F(7,567)=7.74$, $P<0.001$) and a morphine/time interaction ($F(7,567)=4.19$, $P<0.001$), but no nandrolone effect ($F(1,81)=0.86$, NS) and no morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(1,81)=0.01$, $F(7,567)=0.32$ and $F(7,567)=0.51$, respectively, NS).

Similarly, nandrolone co-treatment did not modify the sensitisation to morphine effects on ambulatory movements (Fig. 4C). Three-way ANOVA revealed a morphine effect ($F(1,71)=115.62$, $P<0.001$), a time effect ($F(7,497)=28.44$, $P<0.001$) and a morphine/time interaction ($F(7,497)=27.35$, $P<0.001$), but no nandrolone effect ($F(1,71)=0.04$, NS) and no morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(1,71)=0.42$, $F(7,497)=0.10$ and $F(7,497)=0.16$, respectively, NS). Nandrolone co-treatment also did not modify morphine-induced sensitisation of horizontal activity (Fig. 4D). Three-way ANOVA revealed a morphine effect ($F(1,72)=73.00$, $P<0.001$), a time effect ($F(7,504)=20.25$, $P<0.001$) and a morphine/time interaction ($F(7,504)=22.92$, $P<0.001$), but no nandrolone effect ($F(1,72)=0.11$, NS) and no significant morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(1,72)=2.64$, $F(7,504)=0.43$ and $F(7,504)=0.24$, respectively, NS). We also found no influence of nandrolone

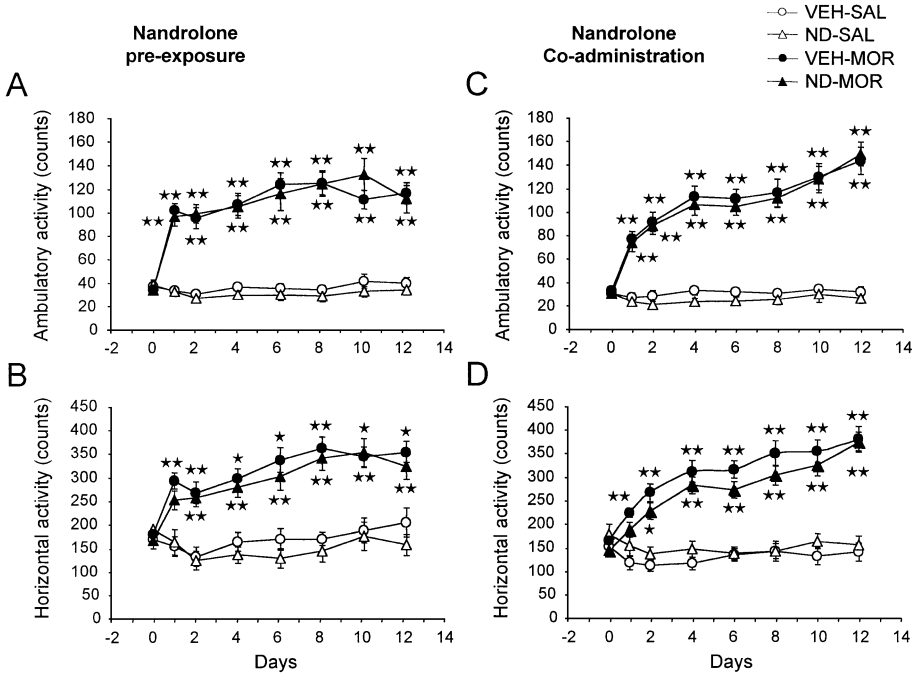

Fig. 4. Sensitisation to locomotor effects induced by repeated morphine administration in mice pre-exposed to (A and B) or co-treated with (C and D) nandrolone (ND) or vehicle (VEH). Mice received repeated morphine (12 mg/kg, s.c., twice a day for 12 days) or saline administration. Ambulatory (AC) and horizontal (BD) activities were recorded 10 min after the daily morning injection, for a period of 10 min. Number of mice per group in the nandrolone co-treatment experiments = 15. Number of mice per group in the nandrolone pre-exposure experiments = 10–16. Data are expressed as means ± S.E.M. $^{\star}P<0.05$, $^{\star\star}P<0.01$ (Dunnett's test, comparison with the respective control basal value on day 0).

co-treatment on the effects of chronic morphine on vertical locomotion (data not shown). Three-way ANOVA revealed a significant time effect ($F(7,504)=6.52$, $P<0.001$), but no morphine effect ($F(1,72)=2.30$, NS), nandrolone effect ($F(1,72)=0.09$, NS), morphine/time, morphine/nandrolone, nandrolone/time and morphine/nandrolone/time interactions ($F(7,504)=1.01$, $F(1,72)=1.52$, $F(7,504)=0.73$ and $F(7,504)=0.71$, respectively, NS).

3.5. Effects of nandrolone treatments on morphine rewarding effects

We explored the influence of nandrolone on morphine rewarding effects by using the conditioned place preference paradigm (Fig. 5). No initial preference or aversion for any compartment was observed in any of the experiments.

The effects of nandrolone pre-exposure were first evaluated (Fig. 5A and B). A significant rewarding effect of morphine (5 mg/kg, s.c.) was observed in the place conditioning paradigm in mice previously receiving intramuscular vehicle for 14 days. This was revealed by a significant increase in the time spent in the drug-paired compartment from the preconditioning to the test phase in the morphine-treated mice (Fig. 5A, Student's t-test, $t(1,12)=-3.28$, $P<0.01$), as well as by significant differences in the score values when those for the morphine-treated mice were

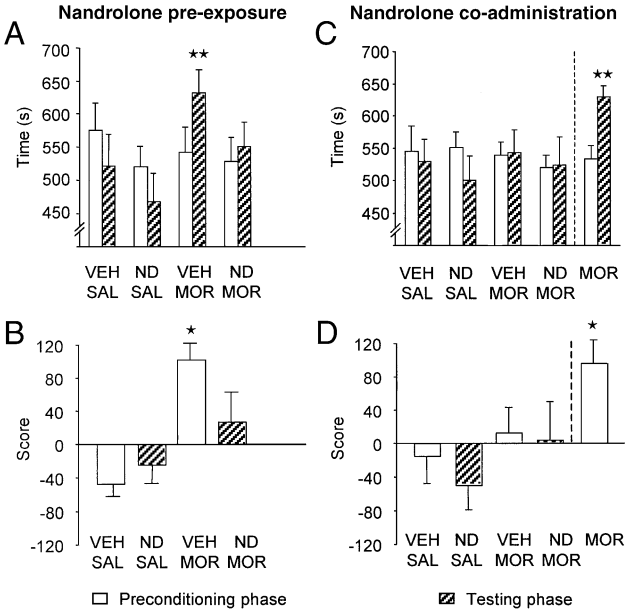

Fig. 5. Rewarding effects of morphine (MOR, 5 mg/kg, s.c.) in the place preference test in mice pre-exposed to (A and B) or co-treated with (C and D) nandrolone (ND) or vehicle (VEH). Control saline (SAL) animals did not receive morphine. The time spent in the morphine-associated compartment during the preconditioning (white bars) and testing (black bars) phases (A and C) and the scores values (B and D) are presented. Number of mice per group in the nandrolone pre-exposure experiments = 12–14. Number of mice per group in the nandrolone co-treatment experiments = 11–14. Values are expressed as means ± S.E.M. $^{\star\star}P<0.01$ (one-way ANOVA, comparison with the respective saline group).

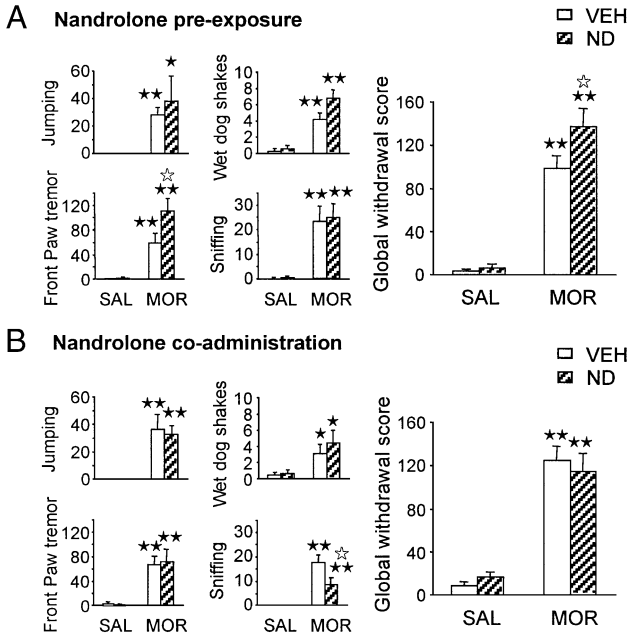

Fig. 6. Number of signs of withdrawal (jumping, wet dog shakes, front paw tremor and sniffing) and global withdrawal score after naloxone administration in morphine (MOR)-dependent mice pre-exposed to (A) or co-treated with (B) nandrolone (ND) or vehicle (VEH). Control saline (SAL) animals did not receive morphine. Abstinence was precipitated by the administration of the opioid receptor antagonist, naloxone (1 mg/kg, s.c.), in mice receiving a chronic treatment of increasing doses of morphine for 6 days. Somatic signs of withdrawal were observed for 30 min immediately after naloxone administration. The global withdrawal score was calculated for each animal by giving each sign a relative weight. Number of mice per group in pre-exposure and co-administration experiments = 9–10. Data are expressed as means ± S.E.M. $^{\star}P<0.05$, $^{\star\star}P<0.01$ (Dunnett's test, comparison with the respective saline control group); $^{\star}P<0.05$ (one-way ANOVA, VEH- vs. ND-treated mice).

compared to those for the respective vehicle group (Fig. 5B, one-way ANOVA, $F(3,49)=3.68$, $P<0.05$, followed by Dunnett's test, $P<0.01$). On the contrary, morphine did not produce any place preference in mice pre-exposed to nandrolone. No difference in the time spent in the drug-paired compartment from the preconditioning to the test phase was found in the morphine-treated mice previously exposed to nandrolone (Student's t-test, $t(1,11)=-0.57$, NS). Moreover, no differences in the scores were observed when those for the morphine-treated mice were compared to those for the respective vehicle control mice (one-way ANOVA, $F(3,49)=3.68$, $P<0.05$, followed by Dunnett's test, NS).

The influence of chronic nandrolone co-administration on morphine rewarding effects was also evaluated (Fig. 5C and D). In our study, the influence of nandrolone co-administration on morphine responses could not be assessed due to the interference of the previous intramuscular injection. Indeed, morphine (5 mg/kg, s.c.) did not produce conditioned place preference when mice received an intramuscular injection of vehicle or nandrolone 1 h before the conditioning session. There were no significant differences in the time spent in the drug-paired compartment from the

preconditioning to the test phase in the morphine-treated mice (Fig. 5C, Student's *t*-test, $t(1,13) = -0.17$, NS, in vehicle group and $t(1,11) = -0.07$, NS, in nandrolone group). Moreover, no differences in the scores were observed when those for the morphine-treated mice were compared to those for the respective vehicle control mice (Fig. 5D, one-way ANOVA, $F(4,57) = 2.86$, $P < 0.05$, followed by Dunnett's test, NS). On the contrary, a significant place preference was observed in a group of mice receiving morphine alone, without any intramuscular co-treatment with vehicle or nandrolone, studied concurrently with the co-administration groups. Thus, a significant increase in the time spent in the drug-paired compartment during the test phase was observed on comparison to the preconditioning value (Student's *t*-test, $t(1,10) = -3.46$, $P < 0.01$), and significant differences in the scores were found when comparing the morphine-treated mice with the respective vehicle control mice (one-way ANOVA, $F(4,57) = 2.86$, $P < 0.05$, followed by Dunnett's test, $P < 0.05$).

3.6. Effects of nandrolone treatments on the naloxone-precipitated morphine withdrawal syndrome

The opioid withdrawal syndrome was precipitated by the administration of naloxone (1 mg/kg, s.c.) in mice receiving chronic increasing doses of morphine for 6 days (Fig. 6). No signs of withdrawal were observed in any group of mice during behavioural observation before the administration of naloxone. Moreover, no behavioural manifestations of withdrawal were observed after the injection of naloxone in saline control mice pre-exposed to or co-treated with nandrolone. Naloxone injection in chronic morphine-treated mice precipitated a withdrawal syndrome manifested by the presence of several somatic signs. The intensity of the syndrome was significantly increased in nandrolone pre-exposed mice (Fig. 6A). Indeed, two-way ANOVA revealed a significant increase of front paw tremor and global withdrawal score (Table 1) in the chronic morphine group pre-exposed to nandrolone in comparison with the group pre-exposed to vehicle. In contrast, the intensity of morphine withdrawal was not modified by the co-administration of nandrolone (Fig. 6B). Indeed, two-way ANOVA (see Table 1) revealed a similar manifestation of jumping, wet dog shakes, front paw tremor, ptosis, teeth chattering, piloerection and body weight loss in chronic morphine-treated mice co-administered with vehicle vs. nandrolone. Only sniffing was significantly reduced in nandrolone co-treated mice. The analysis of the global withdrawal scores also indicated no effect of nandrolone co-treatment on the severity of the morphine withdrawal syndrome (Table 1).

4. Discussion

Pre-exposure to high doses of the anabolic–androgenic steroid nandrolone has been previously reported to induce biochemical changes in the endogenous opioid system in various regions of the brain in rodents (Johansson et al., 1997, 2000a,b; Lukas, 1993; Menard et al., 1995). However, the consequences of such a treatment on morphine-induced behavioural responses related to its addictive properties remained to be examined. In this study, mice were exposed to high doses of nandrolone, comparable to those used by competitive bodybuilders, and the effects on different be-

Table 1
Nandrolone effect on naloxone-precipitated morphine withdrawal

	Two-way ANOVA					
	Morphine	*P*-value	Nandrolone	*P*-value	Interaction	*P*-value
Pre-exposure						
Jumping	$F(1,48) = 13.97$	< 0.001	$F(1,48) = 0.32$	n.s.	$F(1,48) = 0.32$	n.s.
Wet dog shakes	$F(1,48) = 56.32$	< 0.001	$F(1,48) = 4.68$	< 0.05	$F(1,48) = 3.32$	n.s.
Front paw tremor	$F(1,48) = 48.04$	< 0.001	$F(1,48) = 4.71$	< 0.05	$F(1,48) = 4.33$	< 0.05
Sniffing	$F(1,48) = 33.00$	< 0.001	$F(1,48) = 0.06$	n.s.	$F(1,48) = 0.02$	n.s.
Teeth chattering	$F(1,48) = 60.82$	< 0.001	$F(1,48) = 4.04$	n.s.	$F(1,48) = 3.09$	n.s.
Piloerection	$F(1,48) = 133.33$	< 0.001	$F(1,48) = 1.01$	n.s.	$F(1,48) = 0.01$	n.s.
Weight loss	$F(1,48) = 0.15$	n.s.	$F(1,48) = 0.01$	n.s.	$F(1,48) = 0.05$	n.s.
Global withdrawal score	$F(1,48) = 142.69$	< 0.001	$F(1,48) = 5$	< 0.05	$F(1,48) = 7.18$	< 0.05
Co-administration						
Jumping	$F(1,35) = 32.78$	< 0.001	$F(1,35) = 0.07$	n.s.	$F(1,35) = 0.07$	n.s.
Wet dog shakes	$F(1,35) = 11.67$	< 0.01	$F(1,35) = 0.69$	n.s.	$F(1,35) = 0.38$	n.s.
Front paw tremor	$F(1,35) = 31.70$	< 0.001	$F(1,35) = 0.02$	n.s.	$F(1,35) = 0.08$	n.s.
Sniffing	$F(1,35) = 44.87$	< 0.001	$F(1,35) = 5.44$	< 0.05	$F(1,35) = 5.44$	< 0.05
Teeth chattering	$F(1,35) = 32.04$	< 0.001	$F(1,35) = 0.12$	n.s.	$F(1,35) = 0.01$	n.s.
Piloerection	$F(1,35) = 15.62$	< 0.001	$F(1,35) = 0.56$	n.s.	$F(1,35) = 2.44$	n.s.
Weight loss	$F(1,35) = 9.31$	< 0.01	$F(1,35) = 41.82$	n.s.	$F(1,35) = 0.76$	n.s.
Global withdrawal score	$F(1,35) = 124.49$	< 0.001	$F(1,35) = 0.02$	n.s.	$F(1,35) = 0.94$	n.s.

Two-way ANOVA with morphine and nandrolone (between subjects) as factor of variations.
n.s., not significant.

havioural and somatic responses induced by acute and chronic morphine administration were evaluated. Two different protocols of nandrolone administration were used, pre-exposure (repeated administration for two weeks before opioid treatment) and co-administration (concomitant with opioid treatment).

We found no modification of acute morphine-induced antinociception and locomotor responses after both nandrolone pre-exposure and co-administration. On the contrary, nandrolone co-administration, which did not produce any intrinsic effect on body temperature, increased the hypothermia induced by the highest dose of morphine used in this study. In agreement with our results, nandrolone has been described to act on the systems implicated in the hypothermic effects of morphine. Morphine has complex effects on body temperature in rodents and induces hyperthermia at low doses by acting on mu-opioid receptors whereas high doses of morphine produce hypothermia by acting on hypothalamic kappa-opioid receptors (Benamar et al., 2001; Geller et al., 1983; Xin et al., 1997). High doses of morphine increase plasma levels of antipyretic substances, such as the adrenocorticotropin hormone (ACTH) and corticosterone (Nikolarakis et al., 1989). A role of the *N*-methyl-D-aspartate (NMDA)/nitric oxide (NO) pathway in the hypothermic effects of morphine has also been proposed (Ulugol et al., 2000). It is noteworthy in this context that nandrolone administration has been reported to modify endogenous opioid systems (Johansson et al., 2000a), as well as hypothalamic expression of the NMDA receptor NR1 subunit (Le Grevès et al., 1997) and the circulating levels of corticosterone and ACTH (Schlussman et al., 2000). Our results showing an acute pharmacological interaction between morphine and nandrolone in the co-administration, but not in the pre-exposure protocol, suggest a rapid non-genomic action of nandrolone. Rapid androgen actions have been already described in the brain. Thus, testosterone has been shown to increase the spike frequency of neurons within seconds in the lateral hypothalamus of male rats (Orsini et al., 1985). This kind of rapid effect is likely to be mediated through steroid intracellular receptors, nonclassic steroid receptors (ion-gated neurotransmitter receptors) or through direct action on physicochemical membrane properties (Falkenstein et al., 2000). However, we found that nandrolone was more effective to influence morphine rewarding effects and the somatic expression of morphine withdrawal when it was chronically administered before the start of opioid treatment (see below). This is in line with the traditional model of steroid action on intracellular androgen receptors that subsequently modulate transcription and protein synthesis, thus triggering genomic events finally responsible for delayed effects (Falkenstein et al., 2000).

Tolerance to the antinociceptive effects of morphine is known to be influenced by a considerable number of compounds (see review, Bhargava, 1994). In our study, both nandrolone pre-exposure and co-administration significantly decreased the development of antinociceptive tolerance. Interestingly, nandrolone effects on morphine tolerance were only observed in the hot plate test but not in the tail immersion test. These results are in agreement with results of previous studies showing that, in rhesus monkeys, testosterone propionate treatment for 14 days did not alter morphine antinociception in the warm-water tail-withdrawal test, the equivalent in monkey of the rodent tail immersion test (Negus et al., 2001). The lower degree of morphine tolerance in the jumping response of the hot plate test in mice receiving nandrolone was not related to an indirect nandrolone effect on locomotion. Indeed, nandrolone did not itself produce any change in locomotor activity and did not modify locomotor responses induced by acute or chronic morphine treatment. Since the tail-withdrawal response is predominantly mediated by spinal mechanisms whereas hot plate responses are thought to require the involvement of supraspinal structures (Grossman et al., 1982; Morgan et al., 1989), it can be suggested that nandrolone action on morphine tolerance would involve supraspinal mechanisms. In accordance with such a hypothesis, microinjection of morphine into the rostral ventromedial medulla and the ventrolateral periaqueductal gray in rats produced greater antinociception in males than in females (Boyer et al., 1998; Krzanowska and Bodnar, 1999). The observation that nandrolone treatment modifies the levels of endogenous opioid peptide immunoreactivity in the periacqueductal gray (Johansson et al., 2000a) further supports such a supraspinal steroid action on the development of tolerance to opioid antinociceptive responses.

The influence of nandrolone on opioid sensitisation and rewarding properties was also investigated. Dopamine activity in the nucleus accumbens appears to be critically involved in opioid rewarding and locomotor responses (Di Chiara, 1995; Koob, 1992). Anabolic–androgenic steroids have been found to either directly or indirectly elevate dopamine levels in the nucleus accumbens (Kindlundh et al., 2001) by interacting with the endogenous opioid system in the rat brain (Hallberg et al., 2000; Johansson et al., 1997, 2000a,b; Lukas, 1993; Menard et al., 1995). Nandrolone increases β-endorphin levels in the ventral tegmental area in the male rat brain, which is consistent with an increase in dopaminergic activity, leading to reward and euphoria (Johansson et al., 1997). Surprisingly, in our study, nandrolone pre-exposure was associated with a decrease in morphine-induced place preference and no changes were observed in the sensitisation to morphine locomotor effects after the different nandrolone treatments. A possible change induced by nandrolone in the imbalance of the different endogenous opioid peptides related to reinforcing/dysphoric effects of the opioids (Johansson et al., 2000a) may explain these discrepancies. Thus, nandrolone was reported to elevate levels of the endogenous κ-agonist, dynorphin, in the striatum, which could account for a dysphoric effect (Johansson et al., 2000a), through inhibition of the dopaminergic activity in this area (Steiner and Gerfen, 1998).

The induction of physical dependence and consequent avoidance of an aversive withdrawal state represents one aspect of the motivational impetus maintaining opioid addiction (Koob and Le Moal, 2001). We now found that chronic treatment with nandrolone did not produce any sign of withdrawal after administration of naloxone in saline-treated mice. This result is consistent with the study of Negus et al. (2001) showing no manifestations of withdrawal in the rhesus monkey after injection of naloxone during a chronic testosterone treatment performed in the same way as in our study (once-daily administration of high doses for 14 days). However, clinical studies suggest that prolonged use of high doses of anabolic–androgenic steroids may induce physical dependence in humans (Brower et al., 1991; Pope and Katz, 1994). The experimental animal results suggest that such an anabolic–androgenic steroid dependence does not directly involve the endogenous opioid system and/or cannot be shown under the standard experimental conditions in rodents and monkeys. Recent reports also described a higher incidence of opioid consumption in anabolic–androgenic steroid abusers (Arvary and Pope, 2000; McBride et al., 1996; Wines et al., 1999). Our results showing that chronic nandrolone pre-exposure significantly enhances naloxone-precipitated morphine withdrawal are in accordance with such an observation in humans, and with biochemical studies indicating changes in the endogenous opioid system after chronic anabolic–androgenic steroid exposure (Harlan et al., 2000; Menard et al., 1995; Johansson et al., 1997). In this context, another possible explanation for the discrepancies between animal and clinical studies regarding the ability of nandrolone to induce dependence could be that this compound might enhance the endogenous opioid activity in a way that produces a level of dependence too subtle to be detected in experimental animals, but sufficient to increase the morphine withdrawal syndrome. Thus, a lower degree of opioid dependence was induced after chronic treatment with inhibitors of the enkephalin degrading enzyme, which increase endogenous opioid activity, than after chronic exogenous opioid administration (Maldonado et al., 1990). Morphine dependence is thought to result from complex adaptive changes. It is possible that nandrolone promotes the agonist action of morphine on neuronal systems that undergo plastic changes, thereby increasing withdrawal symptoms. A major involvement of the brain noradrenergic pathways in morphine physical withdrawal, especially the locus coeruleus, has been proposed (Maldonado et al., 1992b). Interestingly, there is a clinical case report of a hyperadrenergic withdrawal syndrome in an anabolic–androgenic steroid abuser after naloxone challenge (Tennant et al., 1988), which could have been due to a decrease of endogenous opioid activity during anabolic–androgenic steroid withdrawal (Kashkin and Kleber, 1989). The stress-responsive hypothalamic–pituitary–adrenal axis also participates in opioid withdrawal (Koob and Le Moal, 2001). It is relevant that anabolic–androgenic steroids may disrupt components of this axis by acting on the expression of proopiomelanocortin and corticotrophin-releasing hormone (CRF) mRNA in the brain and on the circulating levels of corticosterone and ACTH (Schlussman et al., 2000).

Of particular interest are the differential effects of nandrolone pre-exposure on morphine-induced place preference and withdrawal syndrome, i.e., decreased morphine rewarding properties and increased somatic manifestations of abstinence. Such a result is not surprising since different anatomical and neurochemical mechanisms are involved in opioid reward and physical dependence (Maldonado et al., 1992a,b; Van Ree et al., 1999). One possible explanation for this suppression of morphine reward and increased withdrawal syndrome could be that chronic nandrolone treatment would produce long-term changes in reward brain circuits leading to a progressive decrease in the basal hedonic level, which results in an unpleasant state that would render the organism more vulnerable to development of an addictive process (Koob and Le Moal, 2001).

In summary, we have clarified the consequences of nandrolone exposure on the acute and chronic effects of morphine on behaviour in rodents. These new data have identified the functional consequences of the biochemical changes previously reported in the endogenous opioid system after nandrolone administration. Although many aspects of steroids action on drug abuse still require intensive research, our results give further support to suggestions that the growing use of nandrolone by both athletes and nonathletes could lead to relevant public health problems.

Acknowledgements

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