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α_{2A}-Adrenoceptors regulate D-amphetamine-induced hyperactivity and behavioural sensitization in mice

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Abstract

Stimulants, such as D-amphetamine, enhance the release of dopamine in the central nervous system (CNS) and induce locomotor activation in mice. When amphetamine is administered repeatedly, the locomotor activation is progressively increased. This behavioural sensitization may be associated with the development of drug craving, addiction and dependence. Also noradrenergic mechanisms participate in the mediation of the effects of psychostimulants. In this study we show that mice lacking the α_2-adrenoceptor subtype A (α_{2A}-AR knock-out (KO) on C57Bl/6J background) are supersensitive to the acute locomotor effects of D-amphetamine (5 mg/kg) in a novel environment compared to wild-type (WT) control mice. When both genotypes were treated repeatedly with D-amphetamine (2 mg/kg) they developed locomotor hyperactivation (sensitization), but its amplitude was lower in α_{2A}-AR KO mice. Development of hyperactivation was reduced in both genotypes by pretreatment with the selective α_2-adrenoceptor antagonist, atipamezole (1 mg/kg). Acute atipamezole also attenuated the expression of D-amphetamine-induced behavioural sensitization especially in WT mice. Interestingly, α_{2A}-AR KO mice failed to exhibit persistent sensitization after 2 weeks of abstinence from repeated D-amphetamine. Rewarding properties of D-amphetamine, measured by conditioned place preference, were similar in both genotypes. These findings indicate that D-amphetamine-induced acute and sensitized locomotor effects are controlled by α_2-adrenoceptors. Drugs antagonizing the α_{2A}-adrenoceptor subtype may provide a novel approach for reducing drug sensitization and motor complications caused by dopaminergic agents.

Keywords: α_{2A}-adrenoceptor; D-amphetamine; Atipamezole; Locomotor activity; Behavioural sensitization; Knock-out (mouse)

1. Introduction

Dopamine is involved in many behavioural functions, including reward mechanisms, motivation and learning, and control of locomotor activity. The rewarding effects of D-amphetamine-like psychostimulants are believed to be mediated by enhanced dopamine release in the mesolimbic–striatal dopamine system (Wise and Bozarth, 1987). Stimulants induce locomotor hyperactivity in rodents and cause behavioural sensitization after repeated administration (Robinson and Becker, 1986). Sensitization is caused both by enhancement of dopamine release (presynaptic sensitization) and by adaptation of postsynaptic neurons, which become more sensitive to dopamine (Henry and White, 1992). Behavioural sensitization has been suggested to be a useful animal model to investigate the development of drug craving, addiction and dependence in humans (Robinson et al., 1985; Robinson and Berridge, 1993).

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The involvement of the brain noradrenergic system in the behavioural effects of D-amphetamine has been investigated by several groups (Archer et al., 1986; Archer et al., 1985; Juhila et al., 2003; Mohammed et al., 1986; Ogren et al., 1983). Norepinephrine is taken up from the synaptic cleft into presynaptic noradrenergic neurons by the norepinephrine transporter, which is one of the main targets for psychostimulants. Mice genetically lacking norepinephrine transporter were supersensitive to the effects of psychostimulants, and these responses were accompanied by dopamine D_2/D_3 receptor sensitization (Xu et al., 2002). The noradrenergic effects of D-amphetamine-like drugs are partly mediated by α_1-adrenoceptors, since the α_1-adrenoceptor antagonist prazosin reduces D-amphetamine-induced hyperactivity in rats (Blanc et al., 1994; Dickinson et al., 1988). The involvement of α_{1B}-adrenoceptors in control of locomotor and rewarding effects of psychostimulants was recently confirmed in a study on mice lacking this receptor subtype (Drouin et al., 2002). Also the α_2-adrenoceptor antagonists atipamezole atipamezole and idazoxan were found to reduce D-amphetamine-induced behavioural sensitization in mice (Juhila et al., 2003).

α_2-Adrenoceptors mediate a variety of physiological responses and pharmacological effects in the central nervous system (CNS). α_2-AR agonists decrease and antagonists increase norepinephrine release in the CNS via α_2-autoreceptors in noradrenergic cell bodies and nerve endings (Schoffelmeer and Mulder, 1983; Trendelenburg et al., 2001, 1993). α_2-Adrenoceptors are also known to act as heteroreceptors and to regulate the release of other neurotransmitters such as dopamine (Bucheler et al., 2002; Trendelenburg et al., 1994; Whittington et al., 2001; Yavich et al., 1997).

The three α_2-adrenoceptor subtypes, α_{2A}, α_{2B}, and α_{2C}, are widely distributed both in the CNS and in the periphery (McCune et al., 1993; Nicholas et al., 1996, 1993; Scheinin et al., 1994; Wang et al., 1996). The most abundant CNS subtype, α_{2A}-adrenoceptor, is expressed in mice in the locus coeruleus, other brain stem centers, cerebral cortex, hippocampus and spinal cord (Wang et al., 1996). Recent studies on knock-out (KO) mice have confirmed the crucial role of α_{2A}-adrenoceptors in regulation of monoamine release and turnover and in the sedative, analgesic and hypotensive effects of α_2-adrenoceptor agonists (Altman et al., 1999; Ihalainen and Tanila, 2002; Lahdesmaki et al., 2002, 2003; Schramm et al., 2001). The α_{2C}-adrenoceptor subtype is mainly expressed in the mouse striatum, olfactory tubercle, hippocampus and cerebral cortex (Holmberg et al., 2003; Wang et al., 1996). α_{2C}-Adrenoceptor has a role in modulating many aspects of mouse behaviour, such as startle reactivity, aggressive behaviour, amphetamine-induced locomotor hyperactivity, cognitive functions and behavioural despair (Sallinen et al., 1999, 1998a,b, 1997). The expression of α_{2B}-adrenoceptor has not been convincingly demonstrated in the mouse CNS (Bucheler et al., 2002).

We have here assessed the role of α_2-adrenoceptors in D-amphetamine-induced locomotor activation, behavioural sensitization and reward processes. More specifically, we used mice with targeted inactivation of the gene for the α_{2A}-adrenoceptor (α_{2A}-AR KO), and evaluated the effects of acute and repeated D-amphetamine exposure in these mice and their wild-type controls. We also studied the effects of a subtype non-selective α_2-adrenoceptor antagonist, atipamezole, on the responses to D-amphetamine. Atipamezole was chosen to represent this drug class in most of the experiments, because of its high affinity and specificity for α_2-adrenoceptors compared with e.g. yohimbine or idazoxan and various other α_2-adrenoceptor antagonists (Meana et al., 1996; Newman-Tancredi et al., 1998). Atipamezole also has a greater selectivity for α_2- vs. α_1-adrenoceptors than e.g. yohimbine, and it blocks all three α_2-adrenoceptor subtypes with approximately equal potency and penetrates readily into the CNS (Haapalinna et al., 1997; Virtanen, 1989; Virtanen et al., 1989).

2. Materials and methods

2.1. Animals

Experiments were performed with 12- to 20-week-old male mice. The generation of a mouse line with targeted inactivation of the gene encoding the α_{2A}-AR (α_{2A}-AR KO) and its behavioural phenotype has been described previously (Altman et al., 1999; Lahdesmaki et al., 2002). α_{2A}-AR KO mice were backcrossed to C57Bl/6J mice for a minimum of five generations to produce a congenic line. Age-matched wild-type (WT) C57Bl/6J mice of the same genetic background (Jackson Laboratories, Bar Harbor, Maine) were used as control animals. Groups of 10 mice were housed in standard polypropylene cages ($38 \times 22 \times 15$ cm) with free access to standard certified pelleted food (RM1 Maintenance Expanded SQC; Special Diet Services, Essex, UK) and water. Ambient temperature was 22 °C, and a 12:12 h light/dark cycle was maintained with lights on at 6 a.m. All experiments were carried out between 7 a.m. and 5 p.m. All experiments conformed to the European Communities Council Directive 86/609/EEC, and the experiments had approval of the local committee for laboratory animal welfare.

2.2. Drugs

The following compounds were used: D-amphetamine sulphate (Sigma, St. Louis, MO) and atipamezole hydrochloride (Orion Corporation, Orion Pharma, Turku, Finland). Drugs were dissolved in saline (0.9% NaCl) and administered subcutaneously (s.c.) in a volume of 5 ml/kg.

2.3. Open field motility

General activity and stereotypic behaviours were evaluated in a large grey rectangular box ($50 \times 50 \times 25$ cm) and recorded with a video tracking system (Videotrack, Viewpoint, Champagne au Mont D'Or, France). Ambulatory activity was recorded in 5-min periods for 1 h immediately after saline or D-amphetamine (5 mg/kg) administration. Saline or atipamezole (1 mg/kg) was injected 20 min before the challenge. Measured activity parameters were: ambulatory activity, entries into central area (30×30 cm), maximal velocity and inactivity time.

2.4. Locomotor activity boxes

In behavioural sensitization experiments, ambulatory activity was monitored in transparent standard polypropylene animal cages (38 × 22 × 15 cm) with transparent lids and aspen bedding on the floor. The test cages were placed in a photobeam frame system (Photobeam Activity System PAS, Cage Rack, San Diego Instruments, San Diego, CA). A computer control unit registered the interruptions of photobeams from 16 individual cages. Ambulatory activity was recorded as breaks of two consecutive beams. Activity was recorded in 5-min intervals for 1 h immediately after saline or D-amphetamine (2 mg/kg) administration.

2.5. Sensitization procedure

2.5.1. Chronic treatments on Days 1–8

Mice from both genotypes were divided randomly into five chronic treatment groups: A) Chronic Saline + Saline (long-term treated and challenged with saline); B) Chronic Atipamezole + Saline (long-term treated and challenged with saline preceded by atipamezole); C) Chronic Saline + Saline and Amphetamine only on Day 8 (long-term treated with saline and challenged with amphetamine); D) Chronic Saline + Amphetamine (long-term treated and challenged with amphetamine) and E) Chronic Atipamezole + Amphetamine (long-term treated and challenged with amphetamine preceded by atipamezole).

Saline or atipamezole (1 mg/kg) was administered 20 min before saline or D-amphetamine injections. All mice received two injections every administration day. On the day preceding the start of the injections, the mice were habituated to the test environment by just placing them in the test cages and by measuring locomotor activity during 1 h. Then, the groups of mice were treated with drugs during four consecutive days (Days 1–4) and ambulations were recorded. There were no drug administrations and testing on Days 5 and 6. On Days 7 and 8, the mice were again treated and ambulatory activity was recorded. This treatment schedule has been validated in C57Bl/6J mice (Juhila et al., 2003).

2.5.2. Treatments on Day 9

On Day 9, the effect of atipamezole on the expression of behavioural sensitization was analysed in the mice treated with repeated saline or D-amphetamine. Mice were pre-treated with saline or atipamezole (1 mg/kg) 20 min before D-amphetamine (2 mg/kg) challenge, and ambulatory activity was measured during 1 h.

2.6. Conditioned hyperactivity and persistence of behavioural sensitization

This experiment was carried out in locomotor activity boxes. Until Day 8, the protocol was similar as in the sensitization experiment, and the groups Chronic Saline + Saline, Chronic Atipamezole + Chronic Saline + Amphetamine and Chronic Atipamezole + Amphetamine were chosen for this experiment. Saline or atipamezole (1 mg/kg) was administered 20 min before saline or D-amphetamine (2 mg/kg) challenge, and total locomotor activity (ambulations, fine movements and rearings) was recorded for 1 h on each administration day. Differently from the sensitization procedure, this experiment was extended to Days 15 and 22. On Day 15 after one-week withdrawal, the possible conditioned hyperactivity was measured by injecting all mice in the different chronic groups with saline and measuring total locomotor activity. On Day 22, the level of hyperactivity induced by D-amphetamine was again measured to assess the persistence of drug-induced sensitisation.

2.7. Conditioned place preference

Conditioned place preference was measured in modified black–white boxes. The floor in the black compartment was black plastic and in the white compartment the floor was covered with a steel grid. After the box modification, there was no place preference response in either genotype i.e. the subsequent procedure was unbiased. The size of both compartments was 30 × 30 × 34 cm. The compartments were separated by a guillotine door.

Experiments involved three phases: habituation (Day 1; door open), conditioning (Days 2–7; door closed) and testing (Day 8; door open). Habituation was done to reduce the novelty and stress associated with exposure to the apparatus. Mice were not injected and weighed before the habituation session. Placing a mouse in the middle of the compartment started each session. The animals were allowed to freely explore the environment during 15 min. During the conditioning phase, mice alternatively received D-amphetamine (2 or 4 mg/kg) in one compartment and saline in the other compartment once a day. D-amphetamine or saline were associated equally with both compartments. The place preference testing was done 24 h after the last conditioning trial, and was performed identically compared to the habituation test (15 min without injection and weighing). Time spent on the white compartment was recorded (s) and difference between conditioned subgroups was compared.

2.8. Data analysis

The results are presented as means ± S.E.M. Statistical analyses were carried out using SPSS for Windows 11.0 (SPSS Inc., Chicago, IL). Comparisons of all experiments were performed using repeated measures analysis of variance (RM ANOVA). When statistically significant differences ($p < 0.05$) were found, post-hoc comparisons were carried out using the least significant difference test (LSD).

3. Results

3.1. Spontaneous open field behaviour in WT and α_{2A}-AR KO mice

Entries into the central area were decreased in α_{2A}-AR KO mice in a novel environment ($P < 0.05$; Fig. 1B). No other significant differences were observed between the genotypes in spontaneous locomotion (treatment A; no injection: Fig. 1A, C and D).

3.2. Effects of D-amphetamine and atipamezole on open field activity and stereotypic movements in WT and α_{2A}-AR KO mice

Fig. 1A illustrates the effects of single D-amphetamine (5 mg/kg) and atipamezole (1 mg/kg) injections on the ambulatory activity (treatments C, D, and E). Ambulatory activity was significantly increased by D-amphetamine treatment [D-amphetamine $F_{(1,52)} = 285.32$, $p < 0.001$; genotype: $F_{(1,52)} = 27.28$, $p < 0.001$; D-amphetamine x genotype: $F_{(1,52)} = 45.01$, $P < 0.001$; RM ANOVA] (Fig. 1A). D-amphetamine increased ambulatory activity in both genotypes ($P < 0.001$), but the degree of hyperactivity was greater in

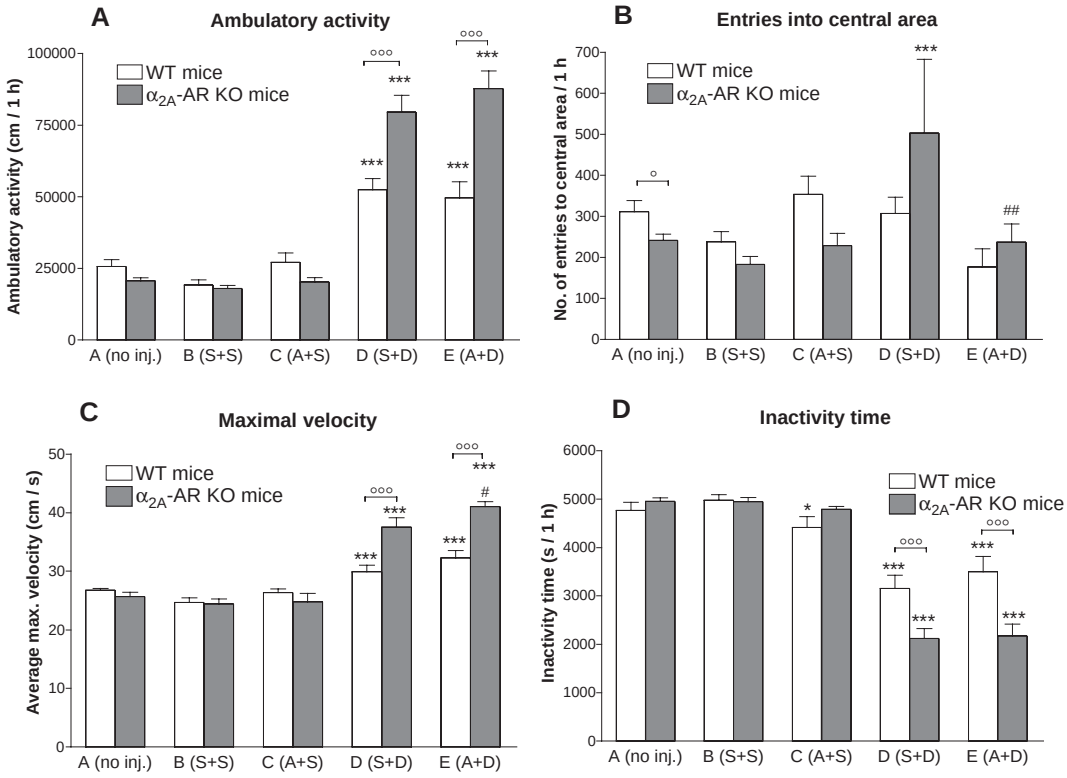

Fig. 1. Open-field behaviour of WT and α_{2A}-AR KO mice after the following acute drug treatments (s.c.) : A) no injection, B) saline+saline, C) atipamezole (1 mg/kg)+saline, D) saline+D-amphetamine (5 mg/kg) and E) atipamezole (1 mg/kg)+D-amphetamine (5 mg/kg). Mice were treated with saline or atipamezole 20 min before saline or D-amphetamine injections. The locomotor responses were measured for 1 h in 5-min periods. Statistical significance of differences between genotypes and treatments is indicated as follows: each treatment group compared to the saline+saline-group (***$p<0.001$, **$p<0.01$ and *$p<0.05$); difference between genotypes ($^{\circ\circ\circ}p<0.001$, $^{\circ\circ}p<0.01$ and $^{\circ}p<0.05$); the effect of atipamezole pretreatment ($^{\#\#\#}p<0.001$, $^{\#\#}p<0.01$ and $^{\#}p<0.05$); (LSD test). Number of mice was 7–10 in all groups.

α_{2A}-AR KO mice ($P<0.001$). Atipamezole pretreatment did not alter D-amphetamine-induced hyperactivity in either genotype.

The number of entries into the central area was significantly increased by D-amphetamine only in the α_{2A}-AR KO mice [D-amphetamine x genotype: $F_{(1,52)}=5.18$, $P<0.05$; D-amphetamine x atipamezole pretreatment: $F_{(1,52)}=8.48$, $p<0.01$; RM ANOVA]. This was abolished by atipamezole pretreatment ($P<0.01$).

The maximal velocity of the mice after D-amphetamine administration was significantly influenced both by pretreatment with atipamezole and by α_{2A}-AR KO genotype [D-amphetamine $F_{(1,52)}=171.58$, $P<0.001$; atipamezole pretreatment: $F_{(1,52)}=6.47$, $p<0.05$; genotype: $F_{(1,52)}=21.98$, $P<0.001$; D-amphetamine x genotype: $F_{(1,52)}=34.84$, $P<0.001$; RM ANOVA] (Fig. 1C). D-amphetamine increased the maximal velocity in both genotypes ($P<0.001$), but the effect was greater in α_{2A}-AR KO mice than in WT mice ($P<0.001$). Atipamezole pretreatment had no effect on velocity in WT mice, but it further increased the velocity of α_{2A}-AR KO mice ($P<0.05$).

Inactivity time was significantly reduced by D-amphetamine treatment [D-amphetamine $F_{(1,52)}=216.25$, $P<0.001$; genotype: $F_{(1,52)}=13.27$, $P<0.001$; D-amphetamine x atipamezole pretreatment: $F_{(1,52)}=4.07$, $P<0.05$; D-amphetamine x genotype: $F_{(1,52)}=23.65$, $P<0.001$; RM ANOVA] (Fig. 1D). D-amphetamine decreased the inactivity time in both genotypes ($P<0.001$), but the α_{2A}-AR KO mice spent less time inactive than WT controls ($P<0.001$). Atipamezole pretreatment before saline challenge also slightly decreased the inactivity time in WT mice ($P<0.05$).

3.3. Development of D-amphetamine-induced behavioural sensitization in WT and α_{2A}-AR KO mice

Fig. 2 illustrates the development of behavioural sensitization after six administrations of D-amphetamine (2 mg/kg) and the effect of atipamezole (1 mg/kg) pretreatment. Development of behavioural sensitization was significantly influenced both by atipamezole pretreatment and by α_{2A}-AR KO genotype [treatment: $F_{(4,350)}=95.51$, $P<0.001$; genotype x treatment: $F_{(4,350)}=10.34$, $P<0.001$; RM ANOVA].

Without D-amphetamine administration there was no significant difference in ambulatory activity between saline- or atipamezole-treated WT and α_{2A}-AR KO mice (treatments A and B). Repeated saline and atipamezole treatment increased activity similarly in both genotypes during the experiment ($P<0.01$).

One day after the habituation session, acute D-amphetamine administration on Day 1 (treatment Day 1) increased ambulatory activity ($P<0.001$) similarly in both genotypes. Chronic treatment with D-amphetamine (treatment Day 8) induced marked locomotor hyperactivity in both genotypes ($P<0.001$), but the level was significantly lower in α_{2A}-AR KO mice ($P<0.001$). Comparison of the groups "Chronic Saline+Saline and Amphetamine only on Day 8" vs. "Chronic Saline+D-Amphetamine" (treatments C and D, Day 8) indicates the development of behavioural sensitization in WT mice ($P<0.001$), but not in α_{2A}-AR KO mice; the acute effect of D-amphetamine on Day 8 was even greater than that produced by the same dose after repeated treatment in α_{2A}-AR KO mice ($P<0.001$).

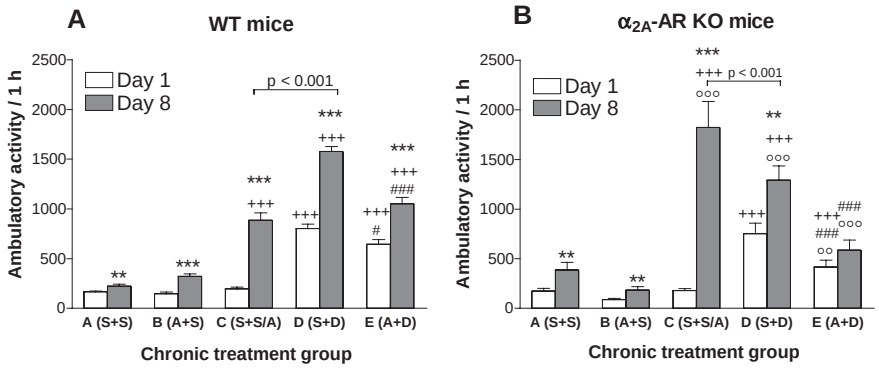

Fig. 2. Development of behavioural sensitization after the following chronic drug treatments (s.c.) in WT and α_{2A}-AR KO mice ($n=19-72$): A) Chronic Saline+Saline, B) Chronic Atipamezole+Saline, C) Chronic Saline+Saline and Amphetamine only on Day 8, D) Chronic Saline+Amphetamine, E) Chronic Atipamezole+Amphetamine. Mice were treated with saline or atipamezole (1 mg/kg) 20 min before saline or D-amphetamine (2 mg/kg) injections. Results represent mean ambulatory activity counts±S.E.M. over 1 h. Statistical significance of differences in: locomotor activity on Day 8 compared to Day 1 in the same treatment group (***$p<0.001$, **$p<0.01$ and *$p<0.05$); locomotor activity of the group compared to the Chronic Saline+Saline −group on the same day ($^{++}p<0.001$, $^{++}p<0.01$ and $^{+}p<0.05$); difference between genotypes ($^{\circ\circ\circ}p<0.001$, $^{\circ\circ}p<0.01$ and $^{\circ}p<0.05$); the effect of atipamezole pretreatment ($^{###}p<0.001$, $^{##}p<0.01$ and $^{#}p<0.05$); (LSD test).

Atipamezole pretreatment (treatment E) decreased acute D-amphetamine-induced ambulatory activity on Day 1 especially in α_{2A}-AR KO mice ($P<0.001$). Atipamezole pretreatment (treatment E) clearly attenuated the development of behavioural sensitization after repeated exposure to D-amphetamine in WT mice on Day 8 ($P<0.001$), and abolished it totally in α_{2A}-AR KO mice ($P<0.001$).

3.4. Effect of atipamezole on the expression of behavioural sensitization after repeated treatment with D-amphetamine

Fig. 3 illustrates the effect of acute atipamezole (1 mg/kg) administration on Day 9 on the D-amphetamine-induced hyperactivity in chronically saline-or D-amphetamine-treated WT and α_{2A}-AR KO mice. The effect of acute D-amphetamine was greater in chronically saline-treated α_{2A}-AR KO mice compared to WT mice ($P<0.01$), suggesting development of sensitization of the α_{2A}-AR KO mice by saline treatment. When compared to the acute D-amphetamine group on Day 8 (Fig. 3A, white panel), chronic D-amphetamine treatment induced behavioural sensitization in WT mice ($P<0.001$), but not at all in α_{2A}-AR KO mice.

Acute atipamezole injection decreased the hyperactivity induced by acute D-amphetamine on Day 8 only in α_{2A}-AR KO mice ($P<0.05$). In chronically D-amphetamine-treated animals, atipamezole reduced the locomotor response to D-amphetamine especially in WT mice ($P<0.001$) and less in α_{2A}-AR KO mice ($P<0.05$) [atipamezole pretreatment: $F_{(1,142)}=16.42$, $P<0.001$; chronic treatment: $F_{(1,142)}=15.93$, $P<0.001$; chronic treatment x genotype: $F_{(1,142)}=24.05$, $P<0.001$; RM ANOVA].

3.5. Conditioned hyperactivity and persistence of the behavioural sensitization after repeated D-amphetamine administration in WT and α_{2A}-AR KO mice

Fig. 4 illustrates total locomotor activity in WT and α_{2A}-AR KO mice after acute (Day 1) and repeated drug treatments (Day 8), after acute saline injection on Day 15 and after acute D-amphetamine injection on Day 22. The locomotor activity depended on the treatment day, chronic treatment and genotype [day: $F_{(3,517)}=46.133$, $P<0.001$; chronic treatment: $F_{(3,517)}=57.20$, $P<0.001$; genotype: $F_{(1,517)}=47.613$, $P<0.001$; RM ANOVA]. There was also an interaction between day x chronic treatment x genotype [$F_{(9,517)}=2.243$, $P<0.05$; RM ANOVA].

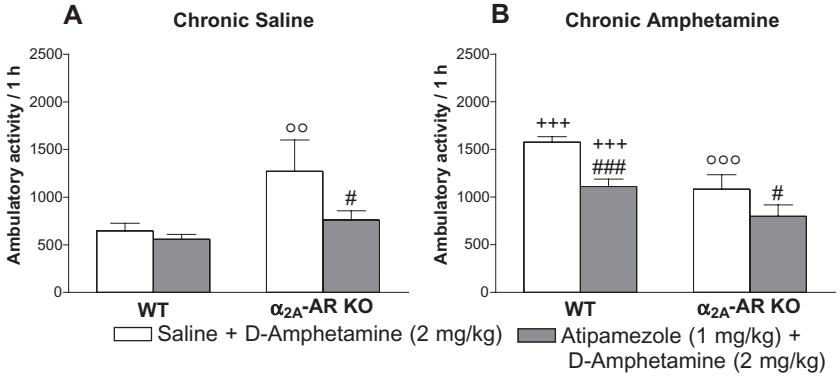

Fig. 3. The effect of atipamezole (1 mg/kg; black bars) pretreatment on the expression of behavioural sensitization to D-amphetamine challenge in chronic saline or D-amphetamine (2 mg/kg) treated WT and α_{2A}-AR KO mice ($n=7-29$). Results represent mean ambulatory activity counts±S.E.M. over 1 h. Statistical significance: the level of behavioural sensitization ($^{+++}p<0.001$, $^{++}p<0.01$ and $^{+}p<0.05$); difference between genotypes ($^{\circ\circ\circ}p<0.001$, $^{\circ\circ}p<.01$ and $^{\circ}p<0.05$); the effect of atipamezole pretreatment ($^{###}p<0.001$, $^{##}p<0.01$ and $^{#}p<0.05$); (LSD test).

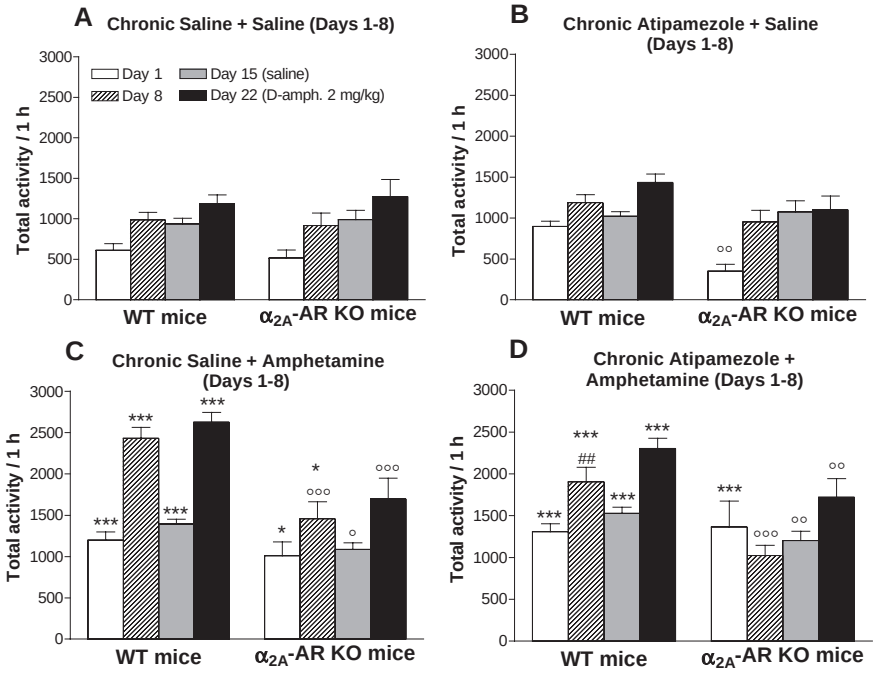

Fig. 4. Conditioned hyperactivity and persistence of behavioural sensitization in repeatedly drug-treated WT and α_{2A}-AR KO mice ($n=12-24$). Treatments during Days 1 to 8 were A) Chronic Saline+Saline, B) Chronic Atipamezole+Saline, C) Chronic Saline+Amphetamine and D) Chronic Atipamezole+Amphetamine, as explained in the behavioural sensitization experiment. On Day 15, the possible conditioned hyperactivity was measured by injecting all mice with saline. On Day 22, the level of hyperactivity after 2 week abstinence was measured after D-amphetamine (2 mg/kg) injection. Results represent mean total activity counts±S.E.M. for 1 h. Statistical significance: difference compared Chronic Saline+Saline group (***$p<0.001$, **$p<0.01$ and *$p<0.05$); difference between genotypes (°°°$p<0.001$, °°$p<0.01$ and °$p<0.05$); the effect of atipamezole pretreatment ($^{###}p<0.001$, $^{##}p<0.01$ and $^{#}p<0.05$); (LSD test).

In chronically saline-treated mice, there were no significant differences between genotypes or treatment days 1 and 8 (Fig. 4A). The activity of the α_{2A}-AR KO mice was lower after acute atipamezole (1 mg/kg) as compared to WT mice ($P<0.01$) (Fig. 4B). No other differences between the genotypes during this assessment were observed.

Repeated administration of D-amphetamine (2 mg/kg) caused hyperactivity in both genotypes, but the amplitude was much lower in α_{2A}-AR KO mice (Fig. 4C: Days 1 and 8). On Day 15, all groups of mice were administered a saline injection to reveal possible conditioned hyperactivity. The total activity was slightly higher in repeatedly D-amphetamine-treated WT mice as compared to the saline-treated group (panel A; $P<0.001$). In α_{2A}-AR KO mice, no hyperactivity was observed on Day 15, thus revealing a significant difference between the genotypes ($P<0.05$). On Day 22, all mice were injected with D-amphetamine (2 mg/kg) to monitor the level of behavioural sensitization after a two-week abstinence. The activity of sensitized WT mice was increased similarly to that of Day 8, and much higher than that seen in acutely D-amphetamine-treated mice from the Chronic Saline+Saline group ($P<0.001$). In α_{2A}-AR KO mice, repeated D-amphetamine administration did not cause similar marked hyperactivity on Day 22. There was also a difference between the genotypes in the repeatedly amphetamine-treated groups ($P<0.001$).

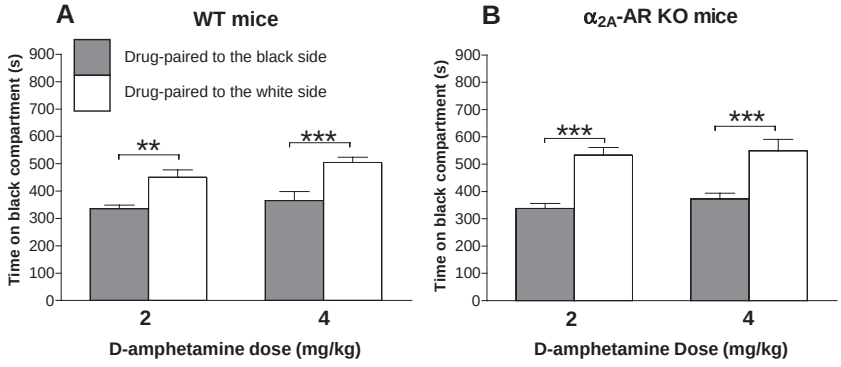

Fig. 5. Conditioned place preference induced by two D-amphetamine doses (2 and 4 mg/kg s.c.) in WT and α_{2A}-AR KO mice ($n=6$). D-Amphetamine was associated equally to both compartments and given either on the first or second day. The place preference test was done 24 h after the last conditioning trial, and was performed identically compared to the habituation test (15 min without injection and weighing). Results represent mean time spent on the white compartment±S.E.M. during 15 min. Statistical significance: difference between conditioning subgroups (***$p<0.001$, **$p<0.01$ and *$p<0.05$); (LSD test).

Fig. 4D shows the effect of atipamezole pretreatment 20 min before D-amphetamine administration. Atipamezole pretreatment decreased the development of sensitization after repeated D-amphetamine administration in WT mice ($P<0.01$). On Days 15 and 22, there were no statistically significant effects of atipamezole pretreatment compared to only amphetamine-treated mice in either genotype. The same significant difference as in only D-amphetamine-treated mice was also found between the genotypes on Days 15 and 22 ($P<0.01$).

3.6. Equal conditioning properties of D-amphetamine in WT and α_{2A}-AR KO mice

Fig. 5 depicts the results of the 15 min conditioned place preference test in WT and α_{2A}-AR KO mice. Conditioning properties of both D-amphetamine doses were analyzed by comparing differences between drug-paired subgroups. The results were examined using three-way ANOVA (genotype x dose x conditioning subgroup). Time spent on the white compartment depended on the conditioning subgroup, but not on dose or genotype [conditioning subgroup: $F_{(1,41)}=71.12$, $P<0.001$; RM ANOVA].

4. Discussion

The findings of the present study indicate that D-amphetamine-induced acute and sensitized locomotor effects are controlled by α_2-adrenoceptors in a complex fashion. While the acute locomotor activating effects of D-amphetamine were increased, the long-lasting behavioural sensitization to repeated D-amphetamine was reduced in α_{2A}-AR KO mice compared to their WT controls. The conditioned rewarding properties of D-amphetamine, as assessed with conditioned place preference, were, however, normally present in the mutant mice.

4.1. Normal spontaneous locomotion and increased acute D-amphetamine-induced hyperactivity in α_{2A}-AR KO mice

Spontaneous locomotor activity was not different in α_{2A}-AR KO mice compared to WT controls. After acute 5 mg/kg D-amphetamine administration, enhanced ambulatory activation, increased velocity and decreased inactivity time were observed in α_{2A}-AR KO mice as compared to WT mice. These findings were very similar to those reported in norepinephrine transporter deficient mice (Xu et al., 2000). These changes could be related to increased brain norepinephrine release in the α_{2A}-AR KO mice (Lahdesmaki et al., 2002, 2003). These findings suggest that α_{2A}-adrenoceptors are not essential in the control of normal locomotion, but their activation by released norepinephrine attenuates D-amphetamine-induced hyperactivity.

Anxiousness and enhanced autonomic functions of the α_{2A}-AR KO mice phenotype have been reported previously (Lahdesmaki et al., 2002; Schramm et al., 2001). Here, the anxiousness of α_{2A}-AR KO mice could also be noticed as decreased entries into the central area of the open field. This possible fear to enter an open area was abolished by D-amphetamine, which could be due to increased ambulatory activation in the α_{2A}-AR KO mice.

4.2. Behavioural sensitization to repeated D-amphetamine is dependent on α_2-adrenoceptor activation

WT mice treated repeatedly with D-amphetamine developed sensitization to the locomotor activation, but in the α_{2A}-AR KO mice the sensitization was not so clear (Fig. 2). Importantly, the level of locomotor hyperactivity was higher in α_{2A}-AR KO mice treated repeatedly with saline and challenged with D-amphetamine on Day 8 than in those treated repeatedly with D-amphetamine (Fig. 2). Furthermore, the level of locomotor hyperactivity was similarly higher in α_{2A}-AR KO mice treated repeatedly with saline and acutely challenged with D-amphetamine on Day 9 than in those α_{2A}-AR KO animals treated repeatedly with D-amphetamine (Fig. 3). This result is consistent with increased sensitivity to the acute locomotor stimulating effect of D-amphetamine (Fig. 1), and also suggests that repeated saline treatment sensitizes the α_{2A}-AR KO mice to acute stimulation by D-amphetamine.

In WT mice, acute and chronic atipamezole attenuated the expression of D-amphetamine-induced sensitization. The smaller sensitization in α_{2A}-AR KO mice was completely blocked by atipamezole (Fig. 3). These results suggest the involvement of α_{2A}-adrenoceptors also in the expression of D-amphetamine sensitization. The sensitization to D-amphetamine was only partly due to conditioning, since in WT mice, but not in the mutants, the response to saline was slightly enhanced after chronic D-amphetamine (Fig. 4).

The WT mice exhibited sensitization to the locomotor activating effect of D-amphetamine that persisted at least two weeks after drug withdrawal. Also, this effect was reduced in the α_{2A}-AR KO mice (Fig. 4). Atipamezole blocked both initial and sustained sensitization in both mouse genotypes, indicating that also other receptor subtypes than α_{2A}-adrenoceptor are involved in these processes. The long-lasting sensitization could be an important factor associated with the high relapse tendency to stimulant abuse in humans (Kalivas et al., 1998), and this may be subject to modulation by α_2-adrenoceptor antagonists.

4.3. Rewarding effects of D-amphetamine are not absent in α_{2A}-AR KO mice

The conditioned place preference test is a procedure that is relatively independent of drug-induced locomotor hyperactivity and should reveal both aversive and rewarding properties of drugs in relation to specific environments. Both genotypes exhibited similar preference to D-amphetamine at pharmacologically effective doses. This result dissociates the neuronal pathways involved in D-amphetamine-induced locomotor hyperactivity and sensitization and those involved

in conditioned rewarding properties. Obviously, the present results do not let us to extrapolate to the direct reinforcing actions of D-amphetamine or drug-seeking behaviour, which would need self-administration experiments.

4.4. Dopaminergic plasticity and α_2-adrenoceptors

Many recent observations have documented the role of noradrenergic-dopaminergic interactions in conveying psychostimulant effects in the CNS. Stimulants increase norepinephrine release in the prefrontal cortex, which can be reduced by the α_2-agonist clonidine (Florin et al., 1994). Single-unit recordings from rat substantia nigra and ventral tegmental area have revealed dual effects of D-amphetamine on dopamine neurons: dopamine-mediated inhibition and a non-dopamine-mediated excitation (mediated partly via α_1-adrenoceptors) (Shi et al., 2000). α_1-adrenoceptors have been suggested to control psychostimulant effects in the prefrontal cortex of mouse and rat (Darracq et al., 1998; Drouin et al., 2002). It has also been shown that mice lacking norepinephrine transporter are supersensitive to the effects of psychostimulants (Xu et al., 2000). Our finding of increased acute D-amphetamine sensitivity of α_{2A}-AR KO mice corroborates the above-mentioned findings in the light of enhanced norepinephrine tone in the CNS in the absence of α_{2A}-adrenoceptor regulation (Lahdesmaki et al., 2002). Importantly, D-amphetamine administration to α_{2A}-AR KO mice results in marked depletion of brain norepinephrine stores (Lahdesmaki et al., 2004).

The ventral tegmental area has been implicated in the development of behavioural sensitization, and its target region nucleus accumbens in the ventral striatum is the main site for the expression of sensitization to psychostimulants (Cador et al., 1999, 1995; Sorg and Ulibarri, 1995). Presynaptic α_2-adrenoceptors located on nerve endings of mesolimbic and nigrostriatal dopaminergic neurons directly regulate dopamine release (Biegon et al., 1992; Bucheler et al., 2002; Sallinen et al., 1997; Trendelenburg et al., 1994; Whittington et al., 2001; Yavich et al., 1997). The localization of immunoreactivity for α_{2C}-adrenoceptors subtypes in dopaminergic cell groups in the substantia nigra and ventral tegmental area suggested that the reported effects of α_2-agonists in the striatum could be at least partly mediated by the α_{2C}-subtype (Lee et al., 1998a,b). Therefore, it cannot be excluded that also α_{2C}-adrenoceptors in these areas are involved in the regulation of the effects of D-amphetamine.

Also other centres in the CNS, such as the prefrontal cortex, may take part in the initiation and expression of the behavioural sensitization induced by D-amphetamine (Cador et al., 1995; Wolf et al., 1995). The importance of the prefrontal cortex in the development of sensitization has been shown (Bjijou et al., 2002; Cador et al., 1999; Drouin et al., 2002). At the receptor level, α_{1B}-adrenoceptors are located close to dopamine D1 receptors, both being present on the same efferent glutamatergic neurons of the prefrontal cortex (Gioanni et al., 1998). In the mouse prefrontal cortex, the release of norepinephrine is mainly regulated by α_{2A}-adrenoceptors and that of dopamine by α_{2C}-adrenoceptors (Ihalainen and Tanila, 2002). It has also been shown that the dopaminergic system in the cortex of sensitized animals is no longer able to inhibit the motor responses to D-amphetamine and cocaine. This change in dopaminergic plasticity in sensitized animals appears to be the result of a qualitative change in the dopamine D2 receptor properties and not the result of a change in the associated γ-aminobutyric acid (GABA) mechanism (Karler et al., 1998). Similar findings were reported in norepinephrine transporter deficient mice, in which supersensitive postsynaptic dopamine D2/D3 receptors were found in striatal slices (Xu et al., 2000). It is possible that changes in norepinephrine release in α_{2A}-AR KO mice might be compensated by a loss of inhibitory control by dopamine autoreceptors either in the prefrontal cortex or striatum. This could partly explain our results, the weakened amplitude of sensitization in α_{2A}-KO mice and the effects of atipamezole pretreatment on initiation and expression of D-amphetamine-induced behavioural sensitization in both genotypes.

The most important noradrenergic nucleus in the brain stem is the locus coeruleus that projects, e.g., to the prefrontal cortex, ventral tegmental area and nucleus accumbens. The locus coeruleus influences the firing activity of midbrain dopamine neurons in the ventral tegmental area and in substantia nigra pars compacta (Grenhoff et al., 1993). Locus coeruleus cell firing and norepinephrine release are under the control of α_2-adrenoceptors (mainly α_{2A}-subtype) (Callado and Stamford, 1999; Jorm and Stamford, 1993). The locus coeruleus is an important area for regulation of vigilance and attention, and for learning and memory (Arnsten et al., 1996, 1998; Coull, 1994; Coull et al., 1995; Granon et al., 1994), but it is also known to participate in mediation of the pharmacological effects of psychostimulants (Kostowski et al., 1977, 1982; Nakai et al., 2002; Ramirez and Wang, 1986). Lesions of the locus coeruleus decrease D-amphetamine-induced locomotion in the rat, and chronic D-amphetamine administration renders the locus coeruleus norepinephrine autoreceptors subsensitive. Our results suggest that α_{2A}-adrenoceptors are important in connecting environmental and drug stimuli, since context-dependent sensitization to D-amphetamine was reduced in the mutant mice. This effect could be directly mediated via several α_2-adrenoceptor subtypes, since it was further reduced by atipamezole.

4.5. Summary

The results of the present study indicate that the brain noradrenergic system is involved in the regulation of D-amphetamine-induced hyperactivity and in the initiation and expression of behavioural sensitization to repeated D-amphetamine administration. The findings also suggest that several α_2-adrenoceptor subtypes are conveying these effects. α_{2A}-adrenoceptors seem to normally antagonize the hyperactivation, and both α_{2A}-and α_{2C}-adrenoceptors are

needed for the development and expression of D-amphetamine-induced sensitization. In the future, subtype-selective α_2-antagonists might be useful in the treatment of stimulant sensitization, but also in other neuropsychiatric disorders, which are related to dopaminergic hyperactivity.

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