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Pharmacology, Biochemistry and Behavior 75 (2003) 373–379
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Dopamine D3 receptor knockout mice and the motivational effects of ethanol

Janel M. Boyce-Rustay*, Fred O. Risinger

Department of Behavioral Neuroscience, L470, Oregon Health Sciences University, 3181 SW Sam Jackson Park Road, Portland, OR 97201-3098, USA

Received 20 September 2002; received in revised form 23 February 2003; accepted 23 April 2003

Abstract

Dopamine D3 receptors have been implicated in the behavioral effects of abused drugs including ethanol. The present experiments characterized the acquisition of ethanol-induced place conditioning and ethanol self-administration in D3 knockout (D3 KO) mice compared with C57BL/6J (C57) mice. For place conditioning, D3 KO and C57 mice received six pairings of a tactile stimulus with ethanol (3 g/kg ip). D3 KO mice showed higher basal locomotor activity levels in comparison with the C57 mice during conditioning. Ethanol produced similar magnitudes of conditioned place preference in both genotypes. In a two-bottle drinking procedure, mice of each genotype received 24 h access to water and either 3% or 10% v/v ethanol. No difference was noted between D3 KO and C57 mice in either consumption or preference. In an operant self-administration procedure using 23 h sessions, D3 KO and C57 mice received access to 10% v/v ethanol on an FR4 schedule of reinforcement, food on an FR1 schedule of reinforcement and water from a sipper tube. D3 KO and C57 mice had similar response rates of ethanol and food as well as similar water intakes. Overall, these results indicate that elimination of D3 receptor function has little influence on ethanol reward or intake.

Keywords: Dopamine D3 receptor; Mice; Ethanol, conditioned place preference; Drinking; Self-administration; Reward, reinforcement

1. Introduction

Dopamine receptor systems often feature prominently in proposed neural mechanisms responsible for drug reward and reinforcement (Koob, 2000; Melichar et al., 2001; Spanagel and Weiss, 1999; Wise, 1998). Dopamine receptors are classified into D1-like (D1 and D5) and D2-like (D2, D3 and D4) receptors (Seeman and Van Tol, 1994). Although D2 and D3 receptors share the D2-like classification, activation of the receptors produce different behavioral responses. For example, (+)-3-PPP, a D2 agonist, increases locomotor activity in rats, whereas pramipexole, a D3 agonist, decreases locomotor activity (Svensson et al., 1994). D2 and D3 receptors also differ in their relative distribution throughout the CNS. D2 receptors are found throughout the brain, with the largest concentrations in the striatum, substantia nigra and ventral tegmental area (Diaz et al., 1995; Gurevich and Joyce, 1999; Landwehrmeyer et al., 1993). On the other hand, D3 receptor distribution is restricted to mesolimbic areas such as the nucleus accumbens, amygdala and olfactory tubercle with minimal overlap with D2 receptors (Diaz et al., 1995; Gurevich and Joyce, 1999; Landwehrmeyer et al., 1993).

Activation or blockade of D3 receptors influences drug self-administration. For example, the D3 agonist PD 128,907 dose-dependently decreases cocaine self-administration in rats, whereas the D3 antagonist nafadotride dose-dependently increases cocaine self-administration in rats (Caine et al., 1997). Pretreatment with BP 897, a D3 partial agonist, dose-dependently decreases operant responding on the cocaine lever in a drug discrimination task in mice (Beardsley et al., 2001). BP 897 pretreatment has no effect on cocaine self-administration in rats but reduces cocaine-seeking behavior in a second-order schedule of reinforcement (Pilla et al., 1999). Dopamine D3 receptors have also been shown to be involved in ethanol reward. U99194A, a dopamine D3 antagonist, enhances ethanol-induced conditioned place preference in Swiss–Webster mice (Boyce and Risinger, 2000, 2002). Furthermore, pretreatment with a D3 agonist or antagonist, respectively, decreases or increases

* Corresponding author. Tel.: +1-503-494-9047; fax: +1-503-494-6877.
E-mail address: boycej@ohsu.edu (J.M. Boyce-Rustay).

0091-3057/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00091-1

ethanol drinking in rats given a two-bottle free choice paradigm (Cohen et al., 1998).

Recently, mice have been created that lack the dopamine D3 receptor (Accili et al., 1996). D3 knockout (D3 KO) mice have no obvious developmental deficits and reproduce normally (Accili et al., 1996). Some investigators have found that D3 KO mice are hyperactive in comparison with wild-type (WT) littermates (Accili et al., 1996) and others have shown no locomotor differences between D3 KO mice and WT mice (Betancur et al., 2000; Boulay et al., 1999). D3 KO mice show increased center entries in an open field test and increased open arm entries and open arm time in the elevated plus maze task in comparison with WT mice (Steiner et al., 1998). Both open field test and elevated plus maze task are used as models of anxiety; therefore, these results suggest that the KO mice are less anxious than WT mice. Although D3 KO mice have increased locomotor activity levels, D3 KO mice still showed more center entries when normalized for activity levels (center entries/crossing) (Steiner et al., 1998). However, a different line of D3 KO mice (maintained on a C57 × SvJ background) showed no difference in measures of anxiety in comparison with WT mice (Xu et al., 1997). In response to cocaine, D3 KO mice show behavioral sensitization to repeated cocaine injections, but this does not differ from WT mice (Betancur et al., 2000).

Preliminary data have also been reported following the administration of ethanol in D3 KO mice. D3 KO mice (of unknown background) are less sensitive to the sedative effects of ethanol and drink more ethanol than the WT mice (Ferreira et al., 2001). On the other hand, D3 KO mice (on a C57 background from the Jackson Laboratory) have been shown to have increased initial sensitivity to ethanol (as measured by an increase in sleep time to a 3.5 g/kg dose of ethanol) in comparison with C57BL/6J (C57) mice but drink less ethanol than C57 mice with a 7% w/v ethanol liquid diet (Narita et al., 2002). The present work extends our previous observations that utilized pharmacological manipulations. These studies indicated that D3 antagonist treatment enhanced ethanol-induced place conditioning but had no effect on ethanol drinking (Boyce and Risinger, 2002). We therefore hypothesized that D3 KO mice would show enhanced acquisition of ethanol-induced conditioned place preference but would not differ from control mice in ethanol drinking behaviors.

2. Methods

2.1. Animals

Male homozygous D3 KO mice (B6.129S4-Drd3^{tm1Dac}) and C57 mice were purchased from the Jackson Laboratory (Bar Harbor, ME) at 7–8 weeks of age. The D3 receptor gene was targeted from stem cells from 129/SvJae and implanted in a pseudopregnant C57 female (Steiner et al., 1998). The chimera offspring were then bred with C57 mice to obtain the D3 KO heterozygous (+/−) mice. The D3 KO heterozygous (+/−) mice were bred and the D3 KO homozygous (−/−) offspring were selected from these heterozygous matings. The D3 KO mice were backcrossed onto a C57 background for five generations. Since the homozygous D3 KO mice (maintained by homozygous sibling matings) were maintained on a C57 background, C57 mice were used as controls in accord with the suggestions given by the Jackson Laboratory. Mice in the place conditioning studies were housed four per cage in polycarbonate cages (27.9 × 9.5 × 12.7 cm) with cob bedding. Mice in the ethanol drinking study were individually housed in stainless steel hanging cages (14 × 18 × 18 cm) with wire mesh fronts and bottoms. Mice in the operant self-administration study were initially housed four per cage in polycarbonate cages. After training, they were housed in mouse operant chambers 23 h/day (see procedure below). For all studies, a 12 h light/dark cycle was in effect (lights on at 0700) and the colony or testing rooms were maintained at an ambient temperature of 21 ± 1 °C. The place conditioning studies were conducted during the light cycle. For the ethanol self-administration study, lever response training was conducted during the light cycle. Lab chow was continuously available in the home cage. Animals in the ethanol self-administration experiment had their access to fluids restricted as described herein. All experimental protocols were approved by the Oregon Health and Science University Animal Care and Use Committee and were in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Guide for the Care & Use of Laboratory Animals, 1996).

2.2. Apparatus

The place conditioning apparatus consisted of eight identical acrylic and aluminum chambers (30 × 15 × 15 cm), each within a ventilated light/sound attenuating box (Med Associates ENV-015 M, St. Albans, VT). Infrared light sources and detectors were positioned opposite each other at 5 cm intervals on the long walls of each chamber, 2.2 cm above the floor surface. Occlusion of the infrared light beams was used both as a measure of locomotor activity and to determine the animal's position in the chamber. Data were recorded each minute by the computer. The floor of each box consisted of interchangeable halves with one of two distinct textures: "hole" floors were made from perforated stainless steel with 6.4 mm round holes on 9.5 mm staggered centers; "grid" floors were composed of 2.3 mm stainless steel rods mounted 6.4 mm apart in Plexiglas rails.

For the operant ethanol self-administration study, lever response training was conducted with four mouse operant chambers (Med Associates Modular Mouse Test Chamber, ENV-307A), each equipped with one ultrasensitive mouse lever (Med Associates ENV-310), liquid dipper with a 0.02 ml cup (Med Associates ENV-303) and 100 mA house

light. The house light was located on the opposite wall from the location of the lever and liquid dipper and was on when a session was active. Each operant chamber was enclosed in a light/sound attenuating cubicle (Med Associates ENV-015M). For 23 h sessions, 16 mouse operant chambers (Med Associates ENV-003) enclosed in light/sound attenuating cubicles were used. Each chamber was equipped with two ultrasensitive mouse levers, liquid dipper with a 0.02 ml cup, 20 mg pellet dispenser (Med Associates ENV-203-20), drinking tube and house light. The access well for the liquid dipper was located in the center of the right side panel. The access well for the pellet dispenser was located in the center of the left panel. The levers were placed on the left side of the liquid dipper well and pellet dispenser. The drinking tube (25 ml glass graduated cylinder fitted with a stainless steel drinking spout) was located in the center of the front panel and connected to a contact lickometer (Med Associates ENV-250A). The house light was centered on the left side panel 9.5 cm above the floor. Session parameters and data collection were controlled by computers adjacent to the chambers using Med Associates interface modules.

2.3. Place conditioning procedure

The place conditioning procedure was conducted daily from Mondays to Fridays. The experimental sequence began with a 5 min habituation session, which was intended to reduce the novelty and stress associated with handling, injection and exposure to the apparatus. All subjects received saline (10 ml/kg) and were immediately placed in the conditioning apparatus for 5 min on a smooth floor covered with paper.

For conditioning, D3 KO mice ($n=11$) and C57 mice ($n=12$) mice were randomly assigned to one of two conditioning subgroups ($n=5-6$ per subgroup) and exposed to an unbiased differential conditioning procedure. Conditioning was conducted using a between-group discrimination design (Cunningham, 1993), where conditioning trials consisted of pairings of a distinctive floor after ethanol exposure and pairings of a different floor with saline. Conditioning subgroups within each genotype were matched for exposure to ethanol and floor type and differed only in the specific floor–ethanol relationship (Cunningham, 1993). On alternate days, mice received 3 g/kg ethanol (20% v/v ip) (CS+ sessions) prior to placement on the grid floor (Grid+ subgroup) or the hole floor (Grid− subgroup). Mice received saline (CS− sessions) prior to placement on the other floor type. Presentation of CS+ and CS− sessions was counterbalanced for order of presentation. Eight conditioning sessions (four CS+, four CS−) were given before the first preference test. Four additional conditioning sessions were performed before a second preference test. For each preference test, all subjects received saline injections before placement in the apparatus for a 60 min session with half-grid floor and half-hole floor (left/right position counterbalanced within groups).

2.4. Ethanol drinking procedure

Fluids were presented in 25 ml graduated glass cylinders with stainless steel drinking spouts. Individually housed subjects ($n=7$ D3 KO, $n=7$ C57) received 24 h access to two tubes containing tap water and ethanol mixed in tap water. Water and ethanol intakes were measured each day for 16 consecutive days. For the first 8 days, 3% v/v ethanol was available. For the second 8 days, 10% v/v ethanol was available. Subjects were weighed every 48 h at which time the water and ethanol tubes were refilled. Left/right position of the tubes was counterbalanced for each genotype and reversed every 48 h. Fluid intakes were measured to the nearest 0.1 ml and corrected for spillage and evaporation by comparing fluid loss from two tubes placed on an empty cage.

2.5. Operant ethanol self-administration procedure

During training, subjects received 2 h access to water each day, 4 h after training sessions. Subjects were first trained to lever press for 20% w/v sucrose solution. Initially, one lever press resulted in 10 s access to the dipper cup (i.e., FR1 schedule of reinforcement). During the course of a 10 day training phase, the schedule of reinforcement was gradually increased to FR4 and the dipper access period was reduced to 5 s. When training was complete, the subjects entered a 15 day initiation phase during which an increasing concentration of ethanol was gradually introduced to the sucrose solution. The concentration of sucrose was gradually reduced such that at the end of this phase subjects were receiving access to 10% v/v ethanol in tap water.

Following the initiation phase, subjects ($n=5$ D3 KO, $n=7$ C57) were placed in operant chambers for 23 h sessions. Forty consecutive sessions (7 days/week) were completed with 10% v/v ethanol available from the dipper (FR4), food from the pellet dispenser (20 mg Noyes Formula A pellets, FR1) and water from the drinking tube. Each day, subjects were removed from the chamber for 1 h in order to clean and resupply the chambers. A 12:12 h light/dark cycle was maintained throughout the procedure.

2.6. Data analysis

ANOVA was utilized for all initial comparisons with an α level set at .05. Analyses with repeated measures utilized a Greenhouse–Geisser correction for inflated α. For the floor preference tests in place conditioning, genotype comparisons were based on the time spent on the grid floor type by each conditioning subgroup. For the ethanol drinking study, comparisons were based on fluid intakes, ethanol doses calculated from milliliter ethanol intakes and ethanol preference ratios calculated by dividing milliliter ethanol intake by total fluid intake. For the ethanol self-administration

study, genotype comparisons were based on responses on the ethanol lever, food lever and water intake for the drinking tube. In addition, response microanalysis was conducted using a procedure that temporally defined related sequences of behavior as bouts (cf. Samson et al., 1988). An ethanol bout consisted of four or more dipper presentations with ≤ 2 min between each dipper presentation. A food bout was defined as two or more pellet deliveries within ≤ 2 min.

3. Results

3.1. Place conditioning

Mean ± S.E.M. activity counts per minute for the habituation trial and the first and last CS+/CS − conditioning trials are shown in Fig. 1. D3 KO mice had higher activity levels compared with C57 mice without drug treatment (habituation and CS − trials) and after ethanol (CS+ trials). Activity declined over CS − trials but not over CS+ trials. Thus, ethanol treatment resulted in higher activity levels compared with saline treatment after several trials. Analysis of activity levels during the habituation session yielded reliable effects of Genotype [$F(1,21)=31.1$, $P<.001$]. Analyses of CS+/CS − activity levels for each trial yielded reliable effects of Genotype during each trial [F's$(1,21)\geq 4.5$, P's$<.05$]. Reliable Trial type effects (CS+/CS −) were noted on Trials 3–6 [F's$(1,21)\geq 7.9$, P's$<.02$]. A reliable Genotype × Trial type interaction was only noted on Trial 6 [$F(1,21)=58.5$, $P<.001$]. Analysis of activity over CS+ trials yielded a reliable Genotype effect [$F(1,21)=15.1$, $P<.001$] but not Trial [$F(5,105)=1.7$, $P<.2$] or Genotype × Trial [$F(5,105)=0.7$, $P<.6$]. Analysis of activity over CS − trials yielded reliable effects of Genotype [$F(1,21)=24.2$, $P<.001$] and Trial [$F(5,105)=50.6$, $P<.001$] but not Genotype × Trial [$F(5,105)=1.7$, $P<.2$].

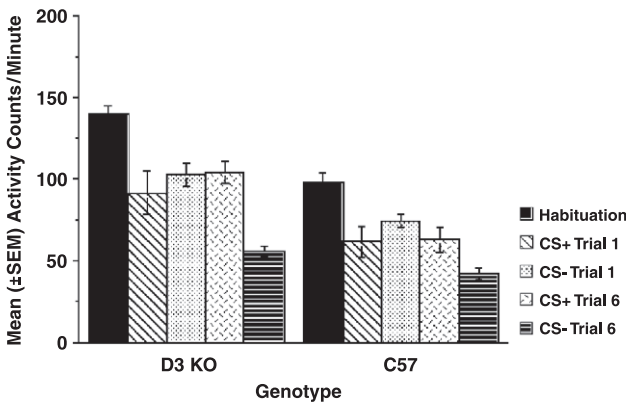

Fig. 1. Mean ± S.E.M. activity counts per minute during the habituation session, CS+ Trial 1, CS − Trial 1, CS+ Trial 6 and CS − Trial 6. On the habituation and CS − trials, subjects received 10 ml/kg ip saline immediately before placement in the conditioning chambers. On CS+ trials, subjects received 3 g/kg ethanol. Duration of all trials was 5 min.

Table 1
Mean ± S.E.M. seconds per minute spent on the grid floor during floor preference testing

Genotype		Conditioning group	
		Grid +	Grid −
Test 1	D3 KO	31.8 ± 2.4	26.3 ± 2.4
	C57	32.1 ± 5.3	26.4 ± 2.9
Test 2	D3 KO	31.5 ± 2.1	23.1 ± 1.9
	C57	38.6 ± 6.5	22.3 ± 2.2

Mean ± S.E.M. seconds per minute on the grid floor type during floor preference testing is given in Table 1. Modest conditioned place preference, as indicated by the between-group comparison between Grid+ and Grid − groups, was noted only on Test 2, which occurred after six conditioning trials. Genotype × Conditioning group analyses yielded a reliable Conditioning group effect on Test 2 [$F(1,19)=10.4$, $P<.004$] but not on Test 1 [$F(1,19)=2.4$, $P<.1$]. Reliable Genotype effects were not seen in either test [F's$(1,19)\leq 0.7$, P's$<.4$]. Reliable Genotype × Conditioning group interactions were also not seen [F's$(1,19)\leq 1.1$, P's$<.3$]. Activity (counts per minute) during the tests were as follows: Test 1, D3 KO 43.3 ± 2.3, C57 33.6 ± 2.2; Test 2, D3 KO 37.1 ± 1.7, C57 26.6 ± 2.5. Reliable Genotype differences in activity were noted in each test [both F's$(1,21)>9.5$, P's$<.006$].

3.2. Ethanol drinking

Water and ethanol intakes are given in Table 2. A procedural error resulted in undetermined water intake on day 14 for three subjects. Minor fluctuations of water or ethanol consumption were noted over days although these were statistically unreliable [water $F(15,135)=2.6$, $P<.07$, ethanol $F(15,150)=2.3$, $P<.09$]. Interactions of day with genotype were not seen [water $F(15,135)=0.5$, $P<.7$, ethanol $F(15,150)=0.7$, $P<.6$]. Therefore, further analyses were based on consumptions collapsed over days within each concentration condition. Water intakes were similar between D3 KO and C57 mice when 3% ethanol was available [$F(1,12)=0.1$, $P<.7$]. When 10% ethanol was available, C57 mice drank less water than D3 KO mice [$F(1,12)=6.6$, $P<.03$]. Ethanol intakes were similar in both ethanol concentration conditions [F's$(1,12)\leq 1.4$, P's$<.2$]. Likewise,

Table 2
Mean ± S.E.M. water intake, ethanol intake, ethanol dose and ethanol preference ratios

Ethanol	Genotype	Water intake (ml)	Ethanol intake (ml)	Ethanol dose (g/kg)	Preference ratio
3% v/v	D3 KO	3.0 (0.3)	4.8 (0.5)	4.1 (0.3)	0.6 (0.1)
	C57	2.8 (0.5)	4.0 (0.3)	3.5 (0.3)	0.6 (0.1)
10% v/v	D3 KO	3.1 (0.4)	3.9 (0.3)	12.1 (1.0)	0.6 (0.1)
	C57	1.8 (0.3)	4.0 (0.3)	11.8 (0.8)	0.7 (0.1)

Table 3
Mean ± S.E.M. responses, intakes and response patterns

	D3 KO	C57
Ethanol responses per session	247 (28)	204 (20)
Food responses per session	290 (27)	251 (12)
Water intake (ml)	3.7 (0.1)	3.5 (0.2)
Ethanol dose (g/kg per session)	3.9 (0.5)	3.0 (0.3)
Ethanol bouts per session	4.6 (0.8)	3.4 (0.5)
Dippers per bout	5.0 (0.4)	4.8 (0.5)
Food bouts per session	52.7 (1.5)	44.1 (1.9)
Pellets per bout	5.3 (0.5)	5.3 (0.2)

ethanol doses were similar for each condition [F's(1,12) $\leq$ 1.7, P's < .2]. No genotype differences were seen with ethanol preference ratios [F's(1,12) $\leq$ 3.4, P's < .09].

3.3. Ethanol operant self-administration

Table 3 gives ethanol lever responding, food lever responding, water intakes, ethanol dose, ethanol bouts per session, dippers per bout, food bouts per session and food pellets per bout averaged over Sessions 6–40. Data from the first five sessions were used for acclimation to the chambers and procedure, and data from these sessions were not used for analysis of genotype differences. Initial repeated-measures analyses were based on Sessions 6–40, with data from each subject averaged over five-session blocks. These analyses revealed no reliable effects of trial block [F's(6,60) $\leq$ 3.0, P's < .07] for either ethanol or food response-related variables. Analysis of water intakes did produce a reliable effect of block [$F(6,60) = 3.8$, $P < .01$]. However, reliable Genotype × Block interactions were not seen with any variable [F's(6,60) $\leq$ 2.3, P's < .1]. Therefore, remaining genotype comparisons were based on data averaged for each subject across Sessions 6–40. These analyses did not reveal reliable effects of genotype for ethanol responding per session [$F(1,10) = 1.6$, $P < .2$], food response [$F(1,10) = 2.3$, $P < .2$], water intake [$F(1,10) = 0.3$, $P < .6$], ethanol dose [$F(1,10) = 2.3$, $P < .2$], ethanol bouts per session [$F(1,10) = 1.8$, $P < .2$], dippers/bout [$F(1,10) = 0.1$, $P < .8$] or food pellets per bout [$F(1,10) = 0.0$, $P < .1$]. However, D3 KO mice did produce higher frequencies of food bouts per session [$F(1,10) = 10.7$, $P < .009$].

4. Discussion

The present results indicate that the mice lacking functional D3 receptors are similar to C57 mice in the acquisition of ethanol-induced conditioned place preference and in ethanol consumption. In part, these results are consistent with the outcome of our previous studies showing pharmacological blockade of D3 receptors did not alter ethanol drinking (Boyce and Risinger, 2002). However, the failure to note genotype differences in ethanol-induced conditioned place preference is not consistent with the results of the pharmacological studies. In those studies, blockade of D3 receptors during acquisition of place conditioning resulted in enhanced ethanol-induced place conditioning (Boyce and Risinger, 2000, 2002). Locomotor activity levels of D3 KO mice were significantly higher than C57 mice both at baseline and after a 3 g/kg ethanol dose. This difference in locomotor activity levels has also been seen in previous studies (Accili et al., 1996; Steiner et al., 1998).

The use of different mouse strains in these studies (i.e., Swiss–Webster mice were used in Boyce and Risinger, 2000, 2002) may also, in part, be responsible for the different pattern of results. The lack of a difference between C57 and D3 KO mice in ethanol-induced place preference is in contrast to the results seen with D-amphetamine. Another line of D3 KO mice that are on a mixed C57/129SvJ background are more sensitive to D-amphetamine and show a significant preference to lower doses of D-amphetamine in comparison with WT mice (Xu et al., 1997). This apparent difference in drug preference may suggest that D3 receptors are not necessary for the acquisition of place preference to ethanol but may be important for other drugs of abuse. On the other hand, it may also suggest that background of the KOs could mask a potential difference between genotypes (cf. Phillips et al., 1999).

C57 mice do not readily acquire ethanol-induced CPP but do acquire amphetamine-induced CPP (Cunningham et al., 1992; MeGeehan and Olive, 2003). Therefore, the background could also account for the differences seen in the sensitivity of ethanol and amphetamine reward between the current study and Xu et al. (1997). This could be due to the inability of the WT C57 strain to form a strong association between the cues and ethanol independent of the genetic mutation (Cunningham et al., 1992). If the D3 KO mice were more sensitive to the rewarding effects of ethanol, we would expect the D3 KO mice to exhibit a significant ethanol-induced CPP on the first test. Other KO models have shown that KO mice show less CPP to ethanol than WT mice (e.g., D2 KO and DARPP-32 KO), but there have been no studies using KOs on a C57 background that exhibited an increased preference for ethanol in comparison with control C57 mice (Cunningham et al., 2000; Risinger et al., 2001).

One preliminary report indicated D3 KO mice drank more ethanol than WT mice (Ferreira et al., 2001). However, our previous study with pharmacological D3 antagonism did not show changes in ethanol drinking (Boyce and Risinger, 2002). The present results also suggest that elimination of D3 receptor action does not influence ethanol intake. The current studies utilized both a 24 h two-bottle choice drinking procedure and an operant self-administration procedure. Both two-bottle intakes (3% and 10% v/v ethanol) and operant responding for 10% v/v ethanol were similar for both strains. Levels of ethanol intakes in C57 mice were in accord with previous reports (Belknap et al., 1993; Risinger et al., 1998). D3 KO and C57 mice showed similar patterns of ethanol responding (i.e., bout size and bout frequency) and did not differ substantially in either food or water intakes.

These D3 KO mice have been previously shown to show a decrease in the total gram per kilogram ethanol intake in a chronic 7% v/v ethanol liquid diet paradigm and had a longer loss of righting reflex to a 3.5 g/kg dose of ethanol, indicating a increase in sensitivity to ethanol (Narita et al., 2002). If the D3 KO mice differed in the sensitivity to the rewarding effects of ethanol, we would have expected to see a difference in the consumption of the 3% v/v ethanol solution. However, D3 KO mice may in fact differ from C57 mice in the ethanol dose response function such that higher or lower ethanol concentrations may have revealed genotype differences.

Overall, the present study suggests that D3 receptor systems may not be important for ethanol reinforcement. D3 receptor involvement in ethanol reward measured via place conditioning remains unclear in that pharmacological blockade of D3 receptor activity enhanced acquisition of ethanol-conditioned place preference (e.g., Boyce and Risinger, 2002). However, that outcome may rely on alternative receptor systems influenced by the pharmacological agent. U99194A (D3 antagonist used in the pharmacological studies) is relatively selective for D3 receptors and is 20-fold more selective for D3 than D2 receptors (Waters et al., 1994). It is still possible that the behavioral results seen with the pharmacological blockade were due to effects at multiple receptor subtypes (D2 and D3 receptors). Furthermore, we did not see a change in locomotor activity. Others have shown that U99194A was able to produce locomotor stimulation on its own that can be blocked by administration of haloperidol (D2 antagonist) (Clifford and Waddington, 1998). On the other hand, discrepancies in the outcomes between studies using pharmacological manipulations and those using KO animals have been seen before. For example, D2 receptor blockade using haloperidol had no influence on the acquisition of ethanol conditioned place preference (Risinger et al., 1992), yet D2 receptor KO mice did not acquire this response (Cunningham et al., 2000).

Future studies utilizing alternative molecular methods (e.g., antisense oligonucleotides and viral-mediated vectors) and inducible KO techniques would address concerns about the influence of possible developmental changes seen with the KOs used in these studies and the role of the D3 receptor in ethanol-mediated behaviors. More studies utilizing both KO mice and pharmacological blockade need to be conducted in order to resolve the contribution strain, genetic background and pharmacological manipulation in the role of D3 receptors in ethanol-mediated behaviors (ethanol-induced CPP and ethanol drinking). In particular, comparison of the present outcome where D3 KO mice did not show enhanced ethanol-induced CPP with results from pharmacological studies using the D3 antagonist U99194A, which enhanced ethanol-induced CPP (Boyce and Risinger, 2002), is difficult due to the different mouse strains used in each set of experiments. Thus, determination of the effect of pharmacological blockade (e.g., using U99194A) on ethanol-induced CPP in C57 mice is needed for a more complete understanding of the failure of D3 KO mice to show enhanced ethanol-induced CPP.

One such study would use the D3 KO and C57 mice given a pretreatment of U99194A during the acquisition of ethanol-induced CPP. If U99194A does not enhance the ethanol-induced CPP in the C57 mice, then the lack of enhancement in the current studies would most likely be due to a strain difference. On the other hand, if the U99194A pretreatment does enhance the ethanol-induced CPP in D3 KO and C57 mice, the effect of the antagonist in the pharmacological studies would be due to effects at a target other than D3 receptors. Another way to resolve the effect of background would be to test the D3 KO mice on the C57 background for amphetamine-induced conditioned place preference. If the D3 KO did not show the increased sensitivity to amphetamine reward, it could also be concluded that the background strain may be masking any potential genetic difference.

Acknowledgements

Supported by NIAAA grants AA10520, AA10760 and AA07468. Christopher Mason and Jeffrey Totten are thanked for assistance in data collection.

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