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α_{1A}- and α_{1B}-Adrenergic Receptors Differentially Modulate Antidepressant-Like Behavior in the Mouse

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Abstract

Tricyclic antidepressant (TCA) drugs are used for the treatment of chronic depression, obsessive compulsive disorder (OCD), and anxiety-related disorders. Chronic use of TCA drugs increases the expression of α_1-adrenergic receptors (α_1-ARs). Yet, it is unclear whether increased α_1-AR expression contributes to the antidepressant effects of these drugs or if this effect is unrelated to their therapeutic benefit. In this study, mice expressing constitutively active mutant α_{1A}-ARs (CAM α_{1A}-AR) or CAM α_{1B}-ARs were used to examine the effects of α_{1A}- and α_{1B}-AR signaling on rodent behavioral models of depression, OCD, and anxiety. CAM α_{1A}-AR mice, but not CAM α_{1B}-AR mice, exhibited antidepressant-like behavior in the tail suspension test and forced swim test. This behavior was reversed by prazosin, a selective α_1-AR inverse agonist, and mimicked by chronically treating wild type mice with cirazoline, an α_{1A}-AR agonist. Marble burying behavior, commonly used to model OCD in rodents, was significantly decreased in CAM α_{1A}-AR mice but not in CAM α_{1B}-AR mice. In contrast, no significant differences in anxiety-related behavior were observed between wild type, CAM α_{1A}-AR, and CAM α_{1B}-AR animals in the elevated plus maze and light/dark box. This is the first study to demonstrate that α_{1A}- and α_{1B}-ARs differentially modulate antidepressant-like behavior in the mouse. These data suggest that α_{1A}-ARs may be a useful therapeutic target for the treatment of depression.

Keywords

alpha 1-adrenergic receptor; depression; tail suspension test; forced swim test; elevated plus maze; obsessive compulsive disorder

1. Introduction

Epinephrine and norepinephrine are important modulators of animal behavior. These catecholamines mediate the “fight or flight” response to an imminent threat, participate in the regulation of mood, regulate feeding behavior, and modulate cognitive function, (see reviews

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by Elhwuegi, 2004; Wellman and Davies, 1991; Lapiz and Morilak, 2006). Abnormalities in adrenergic signaling in the brain are associated with a variety of behavioral pathologies including clinical depression, motor dysfunction, loss of memory, anxiety, and post-traumatic stress disorder (Murchison et al., 2004; Rommelfanger et al., 2007; Dierks et al., 2007). Drugs that inhibit the reuptake or metabolism of norepinephrine and other catecholamines in the central nervous system are widely used in the treatment of depression, obsessive compulsive disorder (OCD), and narcolepsy.

Depression is characterized by subjective feelings of hopelessness, loss of interest in pleasurable activities, sleep disturbances, and fatigue. Evidence from both clinical studies and animal models indicates that adrenergic signaling modulates mood and depression-related behavior. For example, early research showed that the antidepressant efficacy of tricyclic antidepressants (TCA) such as imipramine correlated with inhibition of norepinephrine reuptake (Glowinski and Axelrod, 1964). In addition, selective inhibitors of the norepinephrine transporter such as desipramine and reboxetine exhibit robust antidepressant activity with similar efficacy as that reported for serotonin-selective reuptake inhibitors (SSRIs) when given to patients with major depressive disorder (Bowden et al., 1993; Roth et al., 1990; Nelson, 1999). More recent meta-analysis studies suggest that antidepressants with mixed serotonin-noradrenergic reuptake inhibitor (SNRI) activity may offer therapeutic advantages to treatment with SSRIs alone (Machado et al., 2006; Papakostas et al., 2007, reviewed by Shelton 2004). However, the roles of individual adrenergic receptor (AR) subtypes in modulating depression-related behavior are not well characterized.

The effects of epinephrine and norepinephrine are mediated by adrenergic receptors (ARs). Nine different AR subtypes (α_{1A}-, α_{1B}-, α_{1D}-, α_{2A}-, α_{2B}-, α_{2C}, β_1-, β_2-, β_3-AR) have been cloned and characterized (see review by Strosberg, 1993), and they differ in their amino acid sequences, ligand binding properties, tissue distribution, and coupling to signal transduction pathways. Stone and Quartermain (1999) reported that α_1-AR blockade in the central nervous system induces depression-related behavior in mouse models of depression. In addition, previous studies have reported that administration of TCA drugs increases the density of α_1-ARs in the forebrain, hippocampus, and cerebral cortex of mice and rats (Deupree et al., 2007; Rehavi et al., 1980) and that α_1-ARs in dorsal lateral geniculate neurons, the facial nucleus, and other brain regions become supersensitized following chronic administration of TCA drugs (Menkes and Aghajanian, 1981; Menkes et al., 1983). In contrast, α_2-ARs and β-ARs are downregulated by chronic use of TCA drugs (Deupree et al., 2007; Subhash et al., 2003). However, it has been unclear whether these changes in AR expression and sensitivity actually contribute to the antidepressant effect of these drugs or are only ancillary effects that are not involved in the antidepressant action of TCA drugs. The goal of this study was to investigate the effects of α_{1A}- and α_{1B}-AR signaling on antidepressant-like behavior of the mouse.

The currently available α_1-AR ligands are not sufficiently selective for individual α_1-AR subtypes in vivo to conclusively determine which subtypes modulate behavior. Therefore, we used transgenic mice that express either constitutively active mutant (CAM) α_{1A}- or CAM α_{1B}-ARs (Rorabaugh et al., 2005a) in addition to the endogenous α_{1A}- and α_{1B}-ARs. These mice selectively express CAM α_{1A}- or CAM α_{1B}-ARs only in tissues that normally express the respective wild type receptors (Rorabaugh et al., 2005b; Zuscik et al., 2000). Brains of CAM α_{1A}-AR and CAM α_{1B}-AR mice exhibit a 3-fold and 4.5-fold increase, respectively, in basal inositol-1,4,5-triphosphate production relative to wild type mouse brains, confirming their constitutive activity in vivo (Rorabaugh et al., 2005a; Zuscik et al., 2000). These mice provide a unique tool to investigate the chronic effects of signaling through the α_{1A}- and α_{1B}-AR receptors without the need for subtype-selective drugs.

It has been recently reported that neurogenesis is enhanced in CAM α_{1A}-AR mice (relative to wild type mice) and that this effect can be mimicked by chronically treating wild type mice with cirazoline, an α_{1A}-AR agonist (Gupta et al., 2009). In contrast, CAM α_{1B}-AR signaling induces neurodegeneration (Zuscik et al., 2000). Since several different types of chronic antidepressant therapies are known to induce neurogenesis (Malberg et al., 2000), we investigated the effects of α_{1A}- and α_{1B}-AR signaling on depression-related behavior. Our data provide evidence that α_{1A}-AR signaling, but not α_{1B}-AR signaling, produces antidepressant-like behavior in the mouse.

2. Results

2.1 α_{1A}-AR Signaling, But Not α_{1B}-AR Signaling Causes Antidepressant-Like Behavior

The tail suspension test (TST) is a well established model for the characterization of antidepressant-like behavior (Cryan et al., 2005). We used the TST to determine whether chronically elevated α_{1A}- or α_{1B}-AR signaling promotes antidepressant-like behavior. CAM α_{1A}-AR mice were immobile for significantly less time (44 ± 13 sec) than wild type mice (128 ± 16 sec), suggesting that α_{1A}-AR signaling promotes antidepressant-like behavior (Fig. 1A). In contrast, immobility was slightly increased in CAM α_{1B}-AR mice suggesting that CAM α_{1B}-AR signaling promotes prodepressant-like behavior.

The forced swim test (FST) was used as a second measure of antidepressant-like behavior. CAM α_{1A}-AR mice exhibited significantly less immobility than wild type mice in the FST (Fig. 1B). In contrast, CAM α_{1B}-AR mice exhibited greater immobility than wild type animals. These data are consistent with our observations in the TST, and they further support the conclusion that signaling through α_{1A}-ARs, but not α_{1B}-ARs, promotes antidepressant-like behavior in the mouse.

Locomotor activity of wild type and transgenic mice was measured in an open field to determine whether the differences observed in the TST and FST represent antidepressant/prodepressant-like behavior or are caused by differences in spontaneous motor activity. The distance that CAM α_{1A}-AR mice traveled in the open field was not significantly different from that of wild type mice (Fig. 1C). CAM α_{1B}-AR mice exhibited significantly greater locomotor activity than wild type mice (Fig. 1C) in spite of the fact that they showed increased immobility in the TST and FST. These data suggest that differences in immobility observed between the wild type, CAM α_{1A}-AR, and CAM α_{1B}-AR mice in the TST and FST were not due to generalized differences in spontaneous motility.

2.2 Antidepressant-Like Phenotype of CAM α_{1A}-AR Mice Can be Reversed or Mimicked by Pharmacological Agents

Since an antidepressant-like phenotype was observed in mice expressing CAM α_{1A}-ARs, we hypothesized that this behavior could be blocked by treating CAM α_{1A}-AR mice with an inverse agonist and that this behavior could be mimicked by treating wild type mice with an α_{1A}-AR agonist. We used prazosin, an inverse agonist at constitutively active α_1-ARs (Zhu et al., 2000), to determine whether the antidepressant-like phenotype of CAM α_{1A}-AR mice could be reversed. Intraperitoneal injection of prazosin (0.2 mg/kg) 30 min prior to the TST completely reversed the decreased immobility of CAM α_{1A}-AR mice but had no effect on the immobility of wild type mice (Fig. 2A). We also investigated the effects of chronic cirazoline treatment of wild type mice. This agonist was used because it has 5 to 8-fold greater affinity for the α_{1A}-AR over the α_{1B}- and α_{1D}-AR subtypes, respectively (Horie et al., 1995). In addition, cirazoline is a full agonist at α_{1A}-ARs (E_{max} = 99% of norepinephrine's E_{max}) and only a partial agonist at α_{1B}- and α_{1D}-ARs (E_{max} is approximately 50% for α_{1B}- and α_{1D}-ARs, relative to norepinephrine) (Horie et al., 1995). Mice treated with cirazoline exhibited

significantly decreased immobility in the TST compared to control mice that were not treated with cirazoline (Fig. 2B). Thus, the antidepressant-like phenotype observed in CAM α_{1A}-AR mice is mimicked by treating wild type mice with an α_{1A}-AR agonist. Taken together, these data provide further evidence that the antidepressant-like behavior of CAM α_{1A}-AR mice in the TST is the result of increased α_{1A}-AR signaling.

2.3 α_{1A}-AR Signaling Decreases Marble Burying Behavior

Previous work has demonstrated that serotonin-norepinephrine reuptake inhibitors that are used for antidepressant pharmacotherapy are also effective in the treatment of OCD (Dell'Osso et al., 2006). Marble burying behavior is commonly used as a model of OCD in mice (see review by Witkin, 2008). Since CAM α_{1A}-AR mice exhibited antidepressant-like behavior in the TST and FST, we next examined their behavior in the marble burying assay. CAM α_{1A}-AR mice buried significantly fewer marbles (7.1 ± 1.4) than wild type mice (10.9 ± 0.6) (Fig. 3A). In addition, wild type mice that were chronically treated with cirazoline buried fewer marbles than age matched wild type mice that were not treated with cirazoline (Fig. 3B). Thus, the phenotype observed in the CAM α_{1A}-AR mice can be mimicked by treating wild type mice with an α_{1A}-AR agonist. CAM α_{1B}-AR mice also buried fewer marbles (8.9 ± 0.5) than wild type mice (Fig. 3A), but this difference was not statistically significant. In light of previous studies demonstrating that drugs that reduce marble burying activity in mice are clinically effective in the treatment of OCD (Witkin et al., 2008), our data suggest that the α_{1A}-AR might be a useful therapeutic target for the clinical treatment of OCD.

2.4 α_{1A}-AR and α_{1B} AR Signaling Does Not Effect Anxiety-Related Behaviors

The comorbidity of depression and anxiety is well established, and antidepressant drugs such as tricyclic antidepressants, norepinephrine-selective reuptake inhibitors, and serotonin-selective reuptake inhibitors are clinically used for the chronic treatment of anxiety-related disorders. Therefore, we hypothesized that CAM α_{1A}-AR mice which exhibit antidepressant-like behavior in the TST and FST, may exhibit decreased anxiety-related behavior and that CAM α_{1B}-AR mice (which exhibit prodepressant-like behavior in the TST and FST) may exhibit increased anxiety-related behavior.

The elevated plus maze was used to determine whether α_{1A}-AR signaling affects anxiety. Mice treated with anxiolytic drugs, such as benzodiazepines, spend more time in the open arms of the maze and less time in the closed arms (Walf and Frye, 2007). Both CAM α_{1A}-AR mice and CAM α_{1B}-AR mice spent slightly less time in the open arms and slightly more time in the closed arms compared to wild type mice. However, these differences were not statistically significant (Fig. 4A). Consistent with these results, cirazoline-treated wild type mice spent slightly less time in the open arms and slightly more time in the closed arms compared to age matched wild type animals that were not treated with cirazoline (Fig. 4B).

Light/dark exploration was also used to measure anxiety related behavior. This test is useful because drugs that decrease the amount of time that mice spend in the dark compartment of the box often have anxiolytic effects in humans. We found no differences between CAM α_{1A}-AR mice, CAM α_{1B}-AR mice, or wild type mice with regard to the amount of time that they spent in the dark compartment (Fig. 4C) or the number of entries into the dark compartment (Fig. 4D). In addition, cirazoline had no effect on the amount of time that wild type mice spent in the dark compartment (Fig. 4E) or the number of entries into the dark compartment (Fig. 4F). Taken together, the data from the elevated plus maze and the light/dark box suggest that α_{1A}-AR and α_{1B}-AR signaling do not significantly influence basal levels of anxiety related behavior in the mouse.

3. Discussion

The involvement of norepinephrine in the modulation of antidepressant behavior is well established, and drugs that increase synaptic norepinephrine concentrations by inhibiting norepinephrine reuptake from the synaptic cleft have become important in the treatment of clinical depression. Previous work has demonstrated that α_1-ARs are involved in the antidepressant effects of norepinephrine (Stone and Quartermain, 1999), but the ability of individual α_1-AR subtypes to mediate this antidepressant effect is not well understood. In the present study, we used a unique transgenic mouse model to determine how α_{1A}- and α_{1B}-AR signaling influences antidepressant-like behavior in the mouse. This is the first study to demonstrate that α_{1A}- and α_{1B}-ARs differentially modulate antidepressant-like behavior.

The therapeutic benefit of TCA drugs is typically delayed several weeks following the initiation of drug therapy. This delay is thought to result from changes in the expression of adrenergic and serotonergic receptors in the brain. Previous studies have demonstrated that chronic use of the TCA, imipramine, increases expression of α_1-ARs in the forebrain, hippocampus, and cerebral cortex (Rehavi et al., 1980; Deupree et al., 2007). Nalepa et al. (2002) reported that imipramine or electroconvulsive shock therapy increased the presence of mRNA encoding α_{1A}-ARs, but not α_{1B}-ARs, in the cerebral cortex. However, it has been unclear whether upregulation of α_{1A}-AR expression is directly involved in the antidepressant effect of norepinephrine reuptake inhibitors or is only an ancillary effect that has no role in mediating antidepressant behavior. Our discovery that α_{1A}-AR signaling promotes antidepressant-like behavior in the TST and FST suggests that increased α_{1A}-AR expression following chronic use of norepinephrine-related antidepressants or electroconvulsive shock may play an important role in mediating the antidepressant effects of these therapies.

Previous work has demonstrated that chronic antidepressant therapies including electroconvulsant shock, fluoxetine, tranylcypromine, and reboxetine induce hippocampal neurogenesis (Malberg et al., 2000). Although the mechanism by which α_{1A}-AR signaling promotes antidepressant-like behavior was not characterized in this investigation, recent studies have demonstrated that CAM α_{1A}-AR expression promotes neurogenesis in the mouse (Gupta et al., 2009) and that neurogenesis is also enhanced by chronically treating wild type mice with the α_{1A}-AR agonist, cirazoline (Gupta et al., 2009). In contrast, α_{1B}-AR signaling causes neurodegeneration (Zuscik et al., 2000). Thus, it is quite possible that the antidepressant-like behavior in CAM α_{1A}-AR mice is associated with enhanced neurogenesis, while the prodepressant-like behavior of CAM α_{1B}-AR mice is caused by neurodegeneration. Further work is needed to determine whether there is a causal relationship between neurogenesis and α_{1A}-AR-induced antidepressant-like behavior in these animals as well as the mechanisms involved.

Tricyclic antidepressants and serotonin-norepinephrine reuptake inhibitors that are used clinically to treat depression are also efficacious in the treatment of some patients with OCD (Dell'Osso et al., 2006). Marble burying has been used as a rodent model of OCD (see review by Witkin et al., 2008). In the present study, we found that CAM α_{1A}-AR mice, which exhibit antidepressant-like behavior in the TST and FST, also exhibit decreased marble burying activity. A role for α_{1A}-ARs in the regulation of marble burying behavior is also supported by the observation that marble burying activity was decreased in wild type mice that were chronically treated with cirazoline (Fig. 4B). These data are consistent with the work of Sugimoto et al. (2007) who reported that milnacipran, a serotonin-norepinephrine reuptake inhibitor, decreased marble burying activity in mice. Sugimoto et al. (2007) proposed that the milnacipran-induced decrease in marble burying behavior was caused by enhanced serotonin signaling rather than enhanced adrenergic signaling. However, more recent work has demonstrated that obsessive compulsive-like behavior is also inhibited by reboxetine, a

selective inhibitor of norepinephrine reuptake (Weber et al., 2009). Our data suggest that adrenergic signaling reduces obsessive compulsive-like behavior and that this effect is influenced by α_1-ARs.

α_{1A} – and α_{1B}-ARs are both G_q coupled receptors, and there is significant overlap in the distribution of these receptors in the amygdala, cerebellum, hindbrain, cerebral cortex, and other brain regions (Papay et al., 2006; Day et al., 1997). Despite similarities in their signaling pathways and tissue distributions, there is mounting evidence that the functions of these receptors are not redundant. Studies using transfected cells and isolated tissues have demonstrated that α_{1A}- and α_{1B}-AR subtypes activate divergent signaling pathways that result in different patterns of gene expression and different physiological responses. For example, Gonzalez-Cabrera et al. (2003) found that α_{1A}-AR signaling induces cell cycle arrest in Rat-1 fibroblasts by decreasing the expression of cyclin dependent kinase 6 and increasing the expression of cyclin dependent kinase inhibitor p27. In contrast, α_{1B}-AR signaling induces progression of these cells through the cell cycle (Gonzalez-Cabrera et al., 2003). α_{1A}- and α_{1B}-ARs are also coupled to different signaling pathways in the heart where α_{1A}-ARs, but not α_{1B}-ARs, protect the heart from ischemic injury (Rorabaugh et al., 2005a). Cardiac α_{1A}- and α_{1B}-ARs also differ in their ability to activate pertussis toxin-sensitive signaling pathways that modulate cardiac inotropy (Rorabaugh et al., 2005b). The observation that α_{1A}-AR signaling and α_{1B}-AR signaling differentially modulate behavior in the TST and FST provides further evidence that these α_1-AR subtypes have separate and distinct functions in the central nervous system despite similarities in their anatomical distribution within the brain.

α_{1A}- and α_{1B}–ARs are coexpressed in several brain regions that are known to modulate anxiety-like behavior including the amygdala, hippocampus, prefrontal cortex, and paraventricular nuclei of the hypothalamus (Papay et al., 2006). Several clinical studies have demonstrated that prazosin decreases psychological distress, nightmares, and other anxiety-related symptoms in patients who have post-traumatic stress disorder (PTSD) (Peskind et al., 2003; Raskind et al., 2003), and α_1-AR stimulation also promotes anxiety-related behavior in rats (Handley and Mithani, 1984). These data demonstrate that the anxiety-related symptoms of PTSD are influenced by α_1-ARs. Thus, we were somewhat surprised that anxiety-related behavior was not increased by genetic or pharmacological enhancement of α_1-AR signaling in this study. One limitation of our work is that we only analyzed behavioral indicators of anxiety under basal conditions in which the animals were not subjected to stressful stimuli other than the minimal handling necessary to conduct the experiments. Further work is ongoing to determine whether α_{1A}-or α_{1B}-AR signaling influences anxiety-related behavior in mice subjected to a traumatic event or in mice that have been conditioned to anticipate stress.

In summary, this is the first study to provide direct evidence that α_{1A}- and α_{1B}-ARs are differentially coupled to antidepressant-like behavior in the mouse. Our data further suggest that the α_{1A}-AR subtype may play an important role in mediating the therapeutic effects of TCA drugs that are clinically used in the treatment of depression and OCD. Furthermore, these results suggest a possible role for selective α_{1A}-AR agonists as a novel treatment for depression.

4. Experimental Procedures

4.1 Transgenic mice

B6/CBA mice expressing a constitutively active mutant (CAM) α_{1A}-AR, B6/CBA mice expressing a CAM α_{1B}-AR, and wild type B6/CBA mice were generously donated by Dr. Dianne M. Perez (Cleveland Clinic Foundation, Cleveland, OH). These transgenic mice express constitutively active forms of the α_{1A}- or α_{1B}-ARs in addition to the endogenous wild type α_1-ARs. Generation and genotyping of these mice has been previously described (Rorabaugh et al., 2005a; Zuscik et al., 2000). Briefly, tissue-specific distribution of the CAM

α_{1A}- or CAM α_{1B}-AR was achieved by using the mouse α_{1A}- or α_{1B}-AR promoters to regulate expression of cDNA that encodes a CAM form of the α_{1A}- or α_{1B}-AR, respectively. Approximately 200 copies of the CAM α_{1A}-AR or CAM α_{1B}-AR transgene were injected into the pronuclei of one cell B6/CBA mouse embryos which were implanted into pseudopregnant female mice. Founder mice were identified and subsequent generations were genotyped by southern analysis or polymerase chain reaction using genomic DNA as the template. Tissue-specific distribution of the CAM α_{1A}- and CAM α_{1B}-ARs was confirmed by saturation binding assays with the α_1-AR selective radioligand 2-[β-(4hydroxy-3-[^{125}-I]iodophenyl) ethylaminomethyl]tetralone ([^{125}I]-HEAT) (Rorabaugh et al., 2005a; Zuscik et al., 2000). Constitutive activity of these receptors in the mouse brain and other tissues was determined by measuring basal levels of inositol 1,4,5-trisphosphate production (Rorabaugh et al., 2005a; Zuscik et al., 2000).

Mice were housed with a 12/12 hour light/dark cycle (lights on 0700 – 1900 hours), and all experiments were performed 1200 – 1600 hours. Age matched wild type (n = 84), CAM α_{1A}-AR (n = 98), and CAM α_{1B}-AR (n = 62) mice ages 2 – 6 months were used for all experiments except for mice that were chronically treated with cirazoline, an agonist with 5 to 8-fold selectivity for the α_{1A}-AR versus the α_{1B}- and α_{1D}-ARs, respectively (Horie et al., 1995). Cirazoline-treated mice (n = 20) were continuously administered cirazoline in their drinking water (40 μM) for 9 months starting at the time of weaning and continuing until these experiments were performed. Chronic treatment with cirazoline has been shown to enhance neurogenesis in the mouse (Gupta et al., 2009). Age-matched wild type animals (n = 20) that were not treated with cirazoline were used as a control group for cirazoline-treated animals. Some cirazoline-treated animals were used for multiple experiments.

Approximately equal numbers of male and female mice were used in each experimental group, and no behavioral differences were observed between the two sexes. Food and water were available ad libitum. Animal procedures were approved by the Institutional Animal Care and Use Committee of Ohio Northern University and the University of North Dakota. All experiments using cirazoline treated mice and their age-matched nontreated controls were performed at the University of North Dakota. All other experiments (except elevated plus maze) were performed at Ohio Northern University.

4.2 Tail Suspension Test

The tail suspension test was used to measure antidepressant-like behavior. Mice were individually suspended by the tail from a horizontal bar located 42 cm above the bench top using adhesive tape. Each mouse was suspended for 6 min and recorded with a digital video camera. The amount of time that each mouse remained immobile was later measured by an observer who was blinded to the experimental treatment and mouse genotype.

4.3 Forced Swim Test

The forced swim test was used as an additional measure of antidepressant-like behavior. Mice were given a 15 min pre-swim in a glass cylinder (diameter = 14 cm) containing 15 cm of water (25 °C). Twenty-four hours later, each mouse was placed in the cylinder for 5 min while swimming activity was monitored with a video camera located above the cylinder. The total time that each mouse remained immobile in the water was later measured by an observer who was blinded to the mouse genotype.

4.4 Locomotor activity

Mice were individually placed in the center of a 44 × 44 cm open field for 15 min under ambient light conditions. Locomotor activity was measured using an Opto-M4 Auto-Track System

(Columbus Instruments, Columbus, OH) equipped with 16 lasers (spaced 2.5 cm apart) on each axis. The apparatus was cleaned with ethanol and dried between each mouse.

4.5 Marble Burying Test

The marble burying assay is commonly used as a rodent model of OCD (see review by Witkin, 2008). Each mouse was individually placed in a clear polycarbonate box (18 cm × 28 cm × 13 cm)containing 5 cm of corncob bedding and 15 marbles (3 rows of 5 marbles). The number of buried marbles was counted after 30 min. Marbles were considered buried if they were at least two-thirds covered.

4.6 Elevated Plus Maze

The elevated plus maze was used to measure anxiety. The maze consisted of four Plexiglas arms (30 cm × 5 cm) extending from a common center (5 cm × 5 cm). Two enclosed arms had 13 cm opaque walls, while the center and two open arms had no walls. The maze was positioned 53 cm above the floor. Mice were placed in the center of the maze facing an open arm, and their location (center, open arms, or enclosed arms) was recorded in the absence of investigators by a video camera positioned above the maze. The time spent in each portion of the maze was later measured by an observer who was blinded to the mouse genotype. An entry was defined as having all four paws within the same arm.

4.7 Light/Dark Exploration

The light/dark box was used as an additional measure of anxiety-related behavior. Mice were individually placed in a Plexiglas box (41cm × 33 cm) containing two chambers of equal size (20.5 cm × 16.5 cm). The light chamber had white walls 13 cm high with an open top and was illuminated by a 150 W white light bulb placed 75 cm above the box. The dark chamber had black walls and was enclosed by a lid. A 9 cm × 5 cm opening in the divider between the chambers enabled mice to move between the light and dark chambers. Mice were initially placed in the center of the light chamber facing the opening into the dark chamber and video recorded for 5 min in the absence of investigators using a camera located above the apparatus. The number of entries into the light chamber, number of entries into the dark chamber, and the time spent in each chamber were later measured by an observer who was blinded to the mouse genotype.

4.8 Data analysis

Data are reported as mean ± S.E.M. One-way analysis of variance followed by the Newman-Keuls posthoc test was used for statistical analysis of all experiments except for comparisons between cirazoline treated animals and their age matched controls. The student's t-test was used to analyze cirazoline data because these experiments involved only two groups (cirazoline-treated wild type mice vs. age-matched nontreated wild type mice). Two way analysis of variance was used to compare the effects of water and prazosin in wild type and CAMα_{1A}-AR mice in the tail suspension test since this experiment included two variables (mouse genotype and drug treatment). A value of $p < 0.05$ was considered significant for all analyses.

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Abbreviations

AR
adrenergic receptor

CAM
constitutively active mutant

FST
forced swim test

TCA
tricyclic antidepressant

TST
tail suspension test

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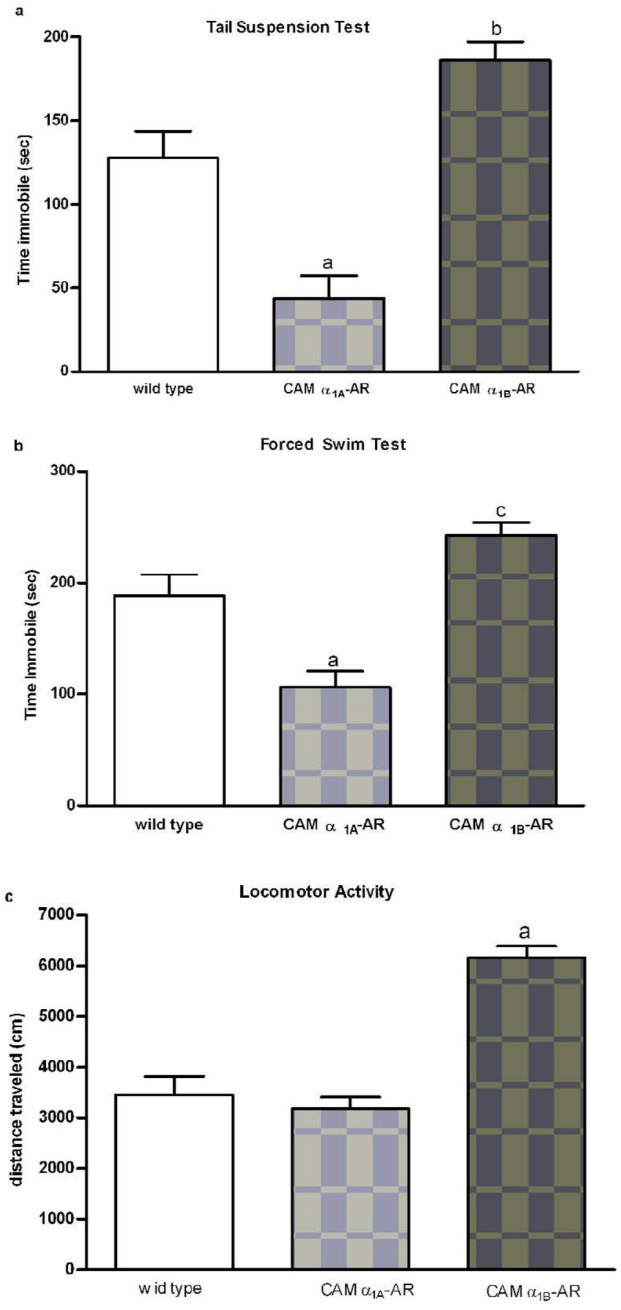

Fig. 1. α_{1A}-AR, but not α_{1B}-AR signaling produces antidepressant-like behavior

Immobility time of CAM α_{1A}-AR mice was significantly decreased [$F = 29.26$ (2,36) $p < 0.001$] and immobility time of CAM α_{1B}-AR mice was significantly increased [$F = 29.26$ (2,36) $p < 0.01$] relative to wild type mice, in the tail suspension test (**Panel A**). Immobility time of CAM α_{1A}-AR mice was significantly decreased [$F = 2.088$ (2,23), $p < 0.001$], while immobility time of CAM α_{1B}-AR mice was significantly increased [$F = 2.088$ (2,23), $p < 0.05$], relative to wild type mice, in the forced swim test (**Panel B**). Locomotor activity in the open field test was significantly greater in CAM α_{1B}-AR mice relative to wild type mice [$F = 29.46$ (2,31), $p < 0.001$] (**Panel C**). Bars represent the mean ± S.E.M of 9 – 15 animals. “a”, “b”, and “c” indicate

a significant difference ($p < 0.001$ and $p < 0.01$, and $p < 0.05$, respectively) compared to wild type mice.

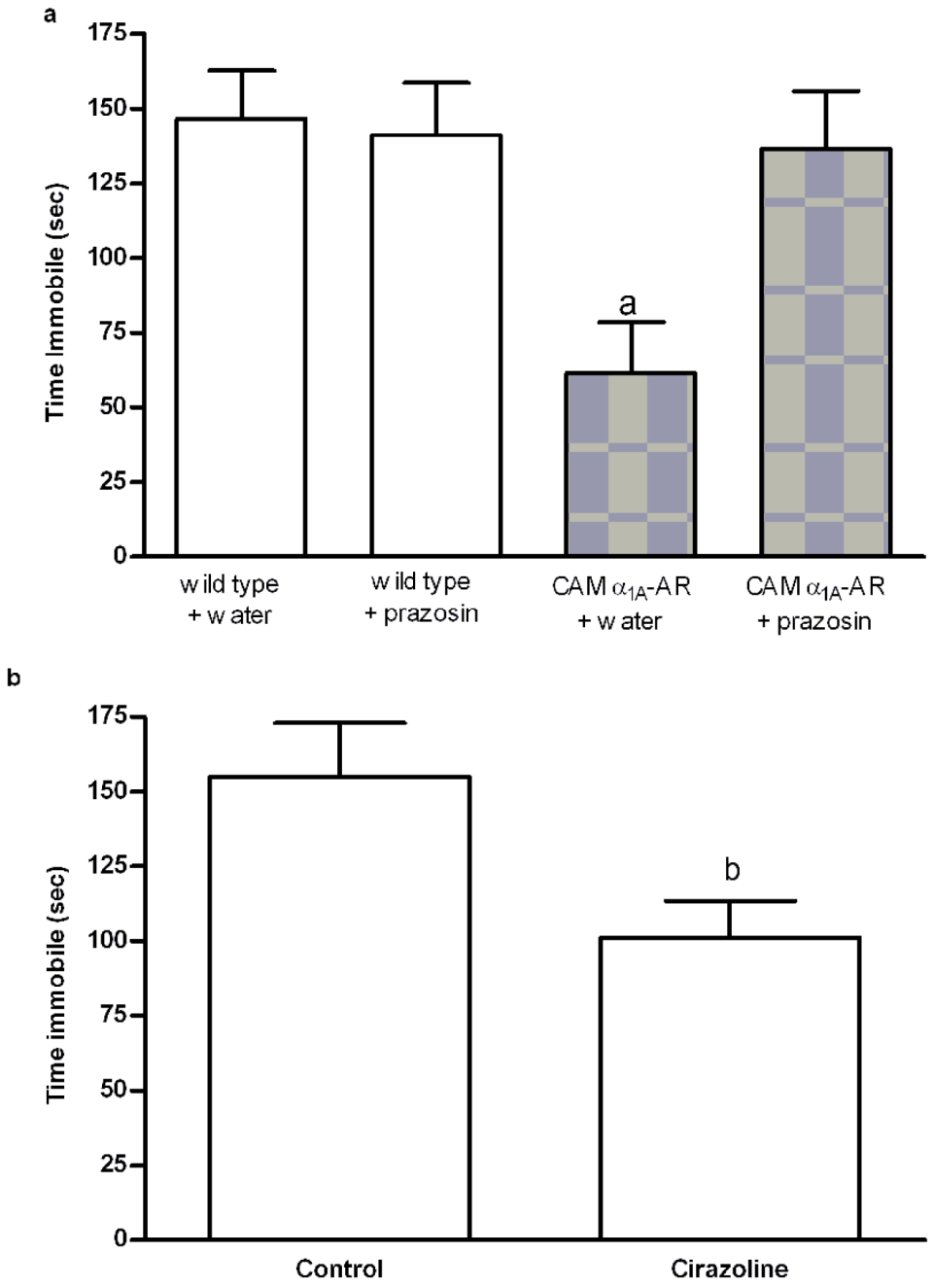

Fig. 2. Antidepressant-like behavior of CAM α_{1A}-AR mice can be reversed by prazosin and mimicked in wild type mice by cirazoline, an α_{1A}-AR agonist

Mice were injected with prazosin or an equal volume of water 30 min prior to the tail suspension test. Prazosin reversed the decreased immobility of CAM α_{1A}-AR mice but had no effect on the immobility of wild type mice (**Panel A**). Data in panel A were analyzed by two way ANOVA using genotype and treatment (water vs. prazosin) as variables. There was a significant effect of prazosin treatment in CAM α_{1A}-AR mice [$F = 5.68$ (1,41), $p < 0.01$]. Wild type mice chronically treated with cirazoline exhibited decreased immobility in the tail suspension test relative to control animals that were not treated with cirazoline (**Panel B**). Data in panel B were analyzed by the student's t test. Bars represent the mean ± S.E.M of 9 - 14

animals. “a” indicates a significant difference ($p < 0.01$) compared to CAM α_{1A}-AR mice treated with prazosin. “b” indicates a significant difference ($p < 0.05$) compared to age matched wild type mice that were not treated with cirazoline.

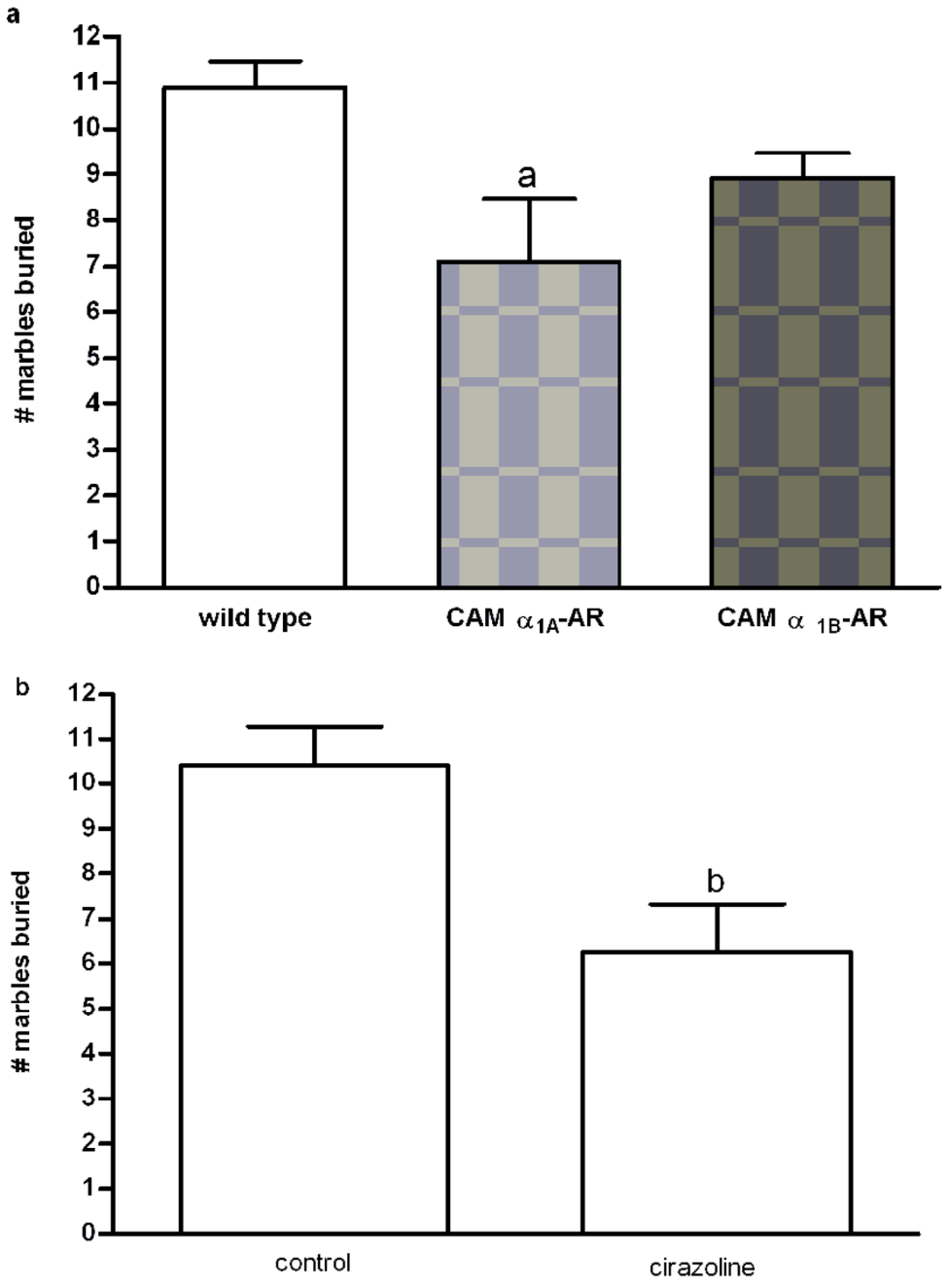

Fig. 3. α_{1A}-AR signaling decreases marble burying behavior

CAM α_{1A}-AR mice buried significantly fewer marbles than wild type mice [F = 4.09 (2,28), $p < 0.05$] (**Panel A**). Wild type mice chronically treated with cirazoline also buried significantly fewer marbles than wild type control mice (**Panel B**). Bars represent the mean ± S.E.M. Data in panel A were analyzed by one way ANOVA. Data in panel B were analyzed by the student's t test. Bars represent the mean ± S.E.M of 9 - 12 animals. "a" indicates a significant difference ($p < 0.05$) compared to wild type animals. "b" indicates a significant difference compared to age-matched wild type mice that were not treated with cirazoline.

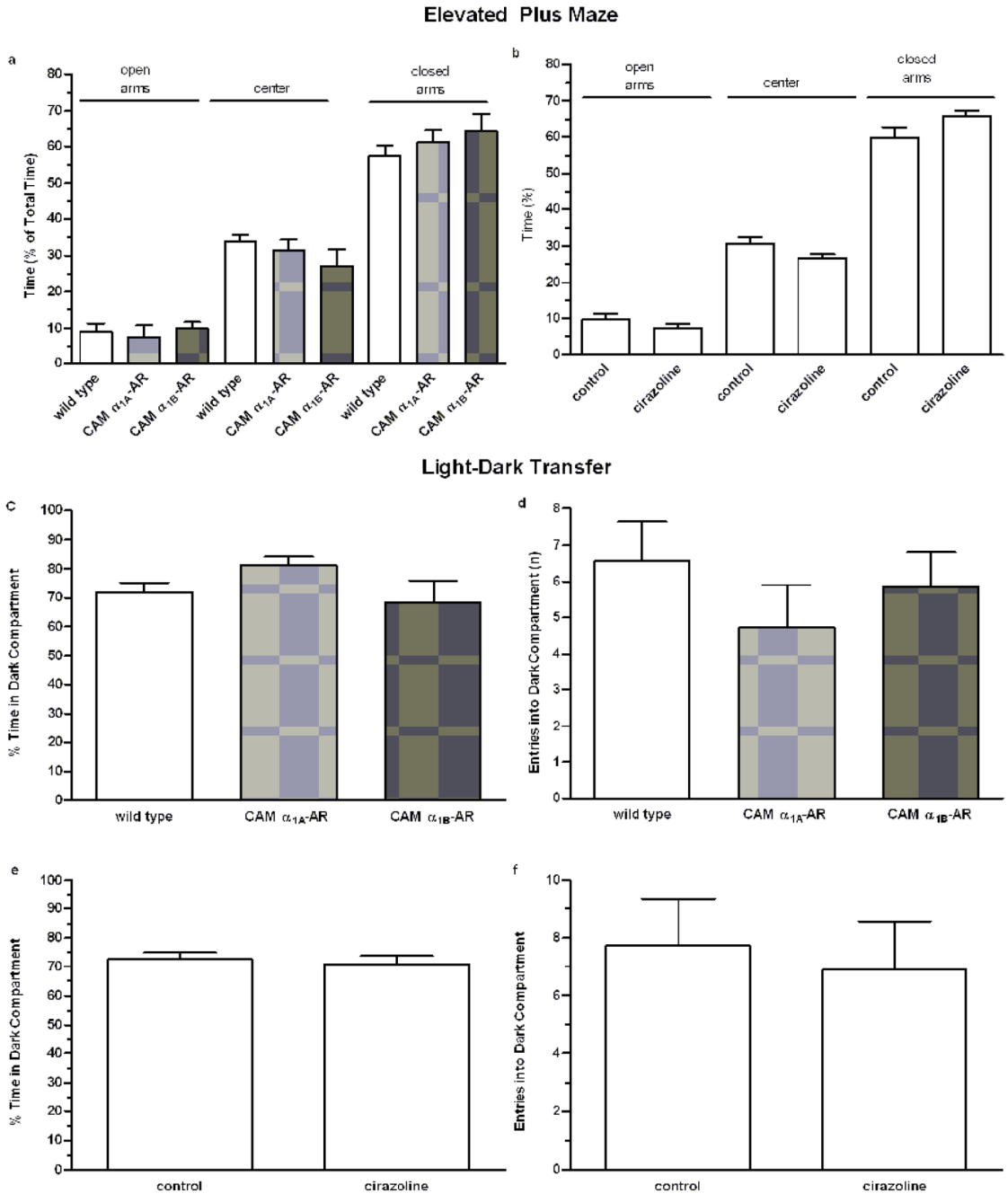

Fig. 4. α_{1A}-and α_{1B}-AR signaling do not alter mouse behavior in the elevated plus maze or light/dark box

CAM α_{1A}-AR mice and CAM α_{1B}-AR mice spent slightly less time in the open arms and slightly more time in the closed arms of the elevated plus maze (**Panel A**). However, these differences were not statistically significant [$F = 0.15$ (2,32), $p > 0.05$]. Likewise, the behavior of wild type mice in the elevated plus maze was not significantly altered by chronic treatment with cirazoline ($p > 0.05$) (**Panel B**). We also observed no significant differences between wild type mice, CAM α_{1A}-AR mice, or CAM α_{1B}-AR mice with regard to the amount of time spent in the dark compartment (**Panel C**) of the light dark box [$F = 2.67$ (2,32), $p > 0.05$] or the number of entries into the dark compartment [$F = 0.77$ (2,32), $p > 0.05$] (**Panel D**). Time in

the dark compartment (**Panel E**) and the number of entries into the dark compartment (**Panel F**) were also unaffected by cirazoline. Bars represent the mean ± S.E.M of 7 – 14 animals.