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RESEARCH ARTICLE

Negative relationship between brain α_{1A}-AR neurotransmission and βArr2 levels in anxious adolescent rats subjected to early life stress

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Abstract

Early-life stress is correlated with the development of anxiety-related behavior in adolescence, but underlying mechanisms remain poorly known. The α_{1A}-adrenergic receptor (AR) is linked to mood regulation and its function is assumed to be regulated by β-arrestins (βArrs) via desensitization and downregulation. Here, we investigated correlation between changes in α_{1A}-AR and βArr2 levels in the prefrontal cortex (PFC) and hippocampus of adolescent and adult male rats subjected to maternal separation (MS) and their relationship with anxiety-like behavior in adolescence. MS was performed 3 h per day from postnatal days 2–11 and anxiety-like behavior was evaluated in the elevated plus-maze and open field tests. The protein levels were examined using western blot assay. MS decreased α_{1A}-AR expression and increased βArr2 expression in both brain regions of adolescent rats, while induced reverse changes in adulthood. MS adolescent rats demonstrated higher anxiety-type behavior and lower activity in behavioral tests than controls. Decreased α_{1A}-AR levels in MS adolescence strongly correlated with reduced time spent in the open field central area, consistent with increased anxiety-like behavior. An anxiety-like phenotype was mimicked by acute and chronic treatment of developing rats with prazosin, an α_{1A}-AR antagonist, suggesting α_{1A}-AR downregulation may facilitate anxiety behavior in MS adolescent rats. Together, our results indicate a negative correlation between α_{1A}-AR neurotransmission and βArr2 levels in both adults and anxious-adolescent rats and suggest that increased βArr2 levels may contribute to posttranslational regulation of α_{1A}-AR and modulation of anxiety-like behavior in adolescent rats. This may provide a path to develop more effective anxiolytic treatments.

Keywords Adolescence · Adults · Anxiety · α_{1A}-Adrenergic receptor · β-Arrestin2 · Early life stress · Hippocampus · Maternal separation · Prazosin · Prefrontal cortex

Abbreviations

PFC	Prefrontal cortex
AR	Adrenergic receptor
GPCR	G protein-coupled receptor
MS	Maternal separation
βArr	β-Arrestin
EPM	Elevated plus-maze
OF	Open field
PZ	Prazosin

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Introduction

There is a growing body of evidence demonstrating that early life stress can increase the risk of developing future psychopathologies, including mood and anxiety disorders (Johnson et al. 2002; Heim et al. 2004; Varese et al. 2012; Vaiserman 2015). Several human and animal studies suggest that early exposure to stressors causes structural and functional alterations in brain areas involved in emotional behavior (Vythilingam et al. 2002; Teicher et al. 2006; Krugers and Joels 2014; Soares-Cunha et al. 2018). For example, early-life adversity was associated with

a reduction in the volume of the prefrontal cortex (PFC) (van Harmelen et al. 2010) and the hippocampus (Rao et al. 2010; Teicher et al. 2012), greater activation of the hypothalamic–pituitary–adrenal axis (Danese and McEwen 2012) and impairment of synaptic plasticity (Herpfer et al. 2012; Chocyk et al. 2013; Bondar and Merkulova 2016; Janthakhin et al. 2017; Rincel et al. 2018). Although mechanisms underlying the consequences of early-life stress are not well-understood, several lines of evidence suggest that susceptibility to stress-induced psychopathology may in part be due to brain noradrenergic dysregulation (Sullivan et al. 1999; Ressler and Nemeroff 2000; Goddard et al. 2010).

Adrenergic receptors (ARs) are a class of G-protein–coupled receptors (GPCRs) and are classified as α_1, α_2, and β. The α_1-ARs are the most abundant ARs in the brain, but their role is the least understood. The α_1-ARs have three subtypes (α_{1A}, α_{1B}, α_{1D}) and mediate multiple physiological impacts of norepinephrine. In rat brain, low levels of the ARs present at birth and reach maximum levels during the first 3 weeks after birth (Morris et al. 1980; Murrin et al. 2007). Stress exposure during this critical period of development has been shown to delay or impair the normal development of (ARs). For example, early-life stress resulted in a delayed development of ARs in cerebral cortex of infant offspring and decreased α_2-AR binding in several brain regions in adult offspring rats (Peters 1984) as well as in the lateral septum of teleost fish (Vindas et al. 2018). Early-life stress has also been shown to reduce α_{1B}-AR binding sites in the cingulate cortex and hippocampus and increase α_{1A} and α_{1B}-AR in the hippocampus of adult mice (Coccurello et al. 2014). Furthermore, early-life stress was found to disturb maternal-infant attachment learning, a type of early learning which is important in shaping behavior in adult (Fillion and Blass. 1986; Sevelinges et al. 2007, 2011; Raineki et al. 2010), in part via an effect on noradrenergic system (Moriceau et al. 2009).

A role of α_1-ARs in mood regulation has been reported in previous studies. For example, the α_{1A}-AR stimulation was found to improve cognitive function, while α_{1B}-AR activation impaired learning and memory (Philipp and Hein 2004). In addition, transgenic mice expressing a constitutively active mutant form of α_{1A}-AR exhibited an antidepressant-like phenotype that was reversed by prazosin, a α_1-AR antagonist (Sevelinges et al. 2007). It has also been shown that antidepressant action of electroconvulsive shock is associated with an increase in the receptor density and mRNA levels of α_{1A}-AR in the rat cerebral cortex and hippocampus (Fillion and Blass 1986). Both α_{1A}-ARs and α_{1B}-AR are expressed in several brain areas involved in the modulation of anxiety-like behavior including the PFC, amygdala, hippocampus, and paraventricular nuclei of the hypothalamus (Nalepa et al. 2002, 2013; Papay et al. 2006). Neurogenesis has been shown to decrease anxiety and depression-related behaviors in a mouse model of stress (Hill et al. 2015). While activation of the α_{1A}-AR has been shown to promote neurogenesis, α_{1B}-AR leads to neurodegeneration (Piascik and Perez 2001). Another study indicated that long-term α_{1A}-AR stimulation leads to decreased depression- and anxiety-like behavior in mice (Doze et al. 2009). Furthermore, tricylic antidepressants, which have been shown to be effective in treating a wide variety of anxiety disorders (Zohar and Westenberg 2000), enhance the α_1-ARs density in the forebrain, hippocampus, and cerebral cortex of rodents (Rehavi et al. 1980; Deupree et al. 2007), suggesting upregulation of α_1-AR expression may be involved in the anxiolytic effect of tricylic antidepressants. α_1-AR antagonist has also been demonstrated to decrease anxiety-related symptoms in patients with post-traumatic stress disorder (Peskind et al. 2003; Raskind et al. 2003). Together, these data suggest a possible role for α_{1A}-AR in anxiety-like behavior.

A major mechanism that controls the responsiveness of GPCRs is homologous desensitization. GPCRs can be desensitized by phosphorylation of the agonist-activated receptor by G-protein-coupled receptor kinase 2. The phosphorylated receptors are then bound by β-arrestins (βArrs), and this interaction in turn blocks further activation of G proteins and downstream signaling pathways and causes receptor internalization (Benovic et al. 1987; Lohse et al. 1990; Oakley et al. 2000, 2001; Laporte et al. 2002; Krasel et al. 2005; Tian et al. 2014; Jean-Charles et al. 2017). Internalized receptors can be recycled to the cell surface (resensitization) or downregulated via degradation in lysosomes (Gainetdinov et al. 2004). Similar to other GPCRs, the α_{1A}-ARs undergo homologous desensitization and internalization following adrenergic agonist stimulation (Hennenberg et al. 2011; Nalepa et al. 2013), which in turn can affect the density of the α_{1A}-ARs and alter their function. There is a growing body of evidence that indicates a role of βArr isoforms in mood regulation. For example, in mice exposed to the HIV-1 transactivator of transcription protein, βArr2 caused μ-opioid-receptor desensitization in amygdala and was associated with enhanced fear and anxiety in the animals (Hahn et al. 2016). Moreover, mice lacking βArr1 or 2 are viable and healthy, but they exhibit altered physiology and behavior compared to wild-type mice (Bjork et al. 2008; Zurkovsky et al. 2017). Previous studies have also indicated that the 17 δ-opioid receptor (δOR; a GPCR) selective agonist SNC80, which has anxiolytic (Saitoh et al. 2004, 2018) and fear-decreasing effects (Saitoh et al. 2004; Li et al. 2009), recruits βArr 1 and 2 (Chiang et al. 2016; Pradhan et al. 2016; Vicente-Sanchez et al. 2018), while the δOR-selective agonist TAN67, which is a weak βArr 2 recruiter, was not associated with a reduction in anxiety-like behavior in mice (van Rijn et al. 2010), suggesting a possible role of

βArr2 in anxiety behavior. In addition, mitogen activated protein kinases, which have been shown to be involved in mood regulation, can scaffold with βArr (Coyle and Duman 2003; Lefkowitz and Shenoy 2005).

In the present study, we attempted to characterize the effects of early-life stress, such as maternal separation (MS), on changes in the expression of the α_1-AR and βArr2 in the PFC and hippocampus of adolescent and adult male rats, and to see if there are correlations between changes in the α_1-AR and βArr2 levels and anxiety-like behavior in developing brain.

Experimental procedure

Pregnant females were housed 4–5 per cage until gestational day 17 and then, were housed in individual cages. The day of birth was considered as day 0. Litters were randomly assigned to one of the two experimental states on day 2: MS ($N=8$) and unhandled (control, $N=8$). MS carried out between postnatal days (PNDs) 2 and 11, a sensitive period for maternal-infant attachment learning, which is important in shaping behavior in adult (Fillion and Blass 1986; Raineki et al. 2010; Sevelinges et al. 2011). Pups were separated from the dam and transferred in a new cage to a different room (to prevent communication with the dam) for 3 h per day (during the light phase, beginning at 8 am) and then were returned to the dam and the home cage. Dams were removed from home cages to a clean cage with ad libitum food and water during MS. During the experiment, the cage was placed on a hot plate set at a temperature of 35 °C, to avoid cooling of rat pups. Litters were weaned at PND 21 and male rats separated from their female littermates. Male pups were group-housed four to five per cage, and food and water were available ad libitum. Rats were housed under controlled environmental states with lights on at 7:00 and controlled temperature and humidity of the room. The behavioral tests were performed in 35–36-day-old male rats (adolescent period). Rats of each group in adolescent rats, after finishing the last behavioral tests, and in adult rats, at PND 62 were killed for western blotting analysis. All procedures were performed in accordance with the National Institutes of animal care and use guidelines approved by the Institutional Ethic Committee (IEC) at Urmia University of Medical Sciences (IR.UMSU.REC.1397.288).

Behavioral tests

At 35–36 day of age, the rat offspring underwent two behavioral tests for anxiety-like behaviors, including elevated plus-maze (EPM) test and open field (OF) test. Behavioral testing was performed in the morning by the investigator blind to the experimental condition under a room light. All test sessions were recorded via a vertically video camera and monitored from an adjacent laboratory.

The OF provides a method for evaluating novel environment exploration, total locomotor activity and anxiety-related behaviors in rodents (Prut and Belzung 2003). To evaluate locomotor activity and anxiety-like behavior, rats were placed individually into a standard OF activity test chamber and were left to explore for 10 min in an arena (40 cm × 40 cm × 35 cm). In the present study, time spent within the central area ($16 \times 16\,cm^2$) of the OF as well as the percentage of distance traveled in the center (calculated by (distance traveled in center/distance traveled in periphery + distance traveled in center) × 100) were measured as indicators of anxiety-like behavior. Total distance traveled in the OF (automatically recorded) , a measure of general locomotor activity, is also interpreted as an anxiety-like response (Sestakova et al. 2013). The chamber was carefully cleaned with ethanol solution after each test and dried between each rat in accordance with previous studies (Seibenhener and Wooten 2015).

The EPM was comprised of two closed arms enclosed by 30 cm high walls (30 × 5 × 5 cm) and two open arms with no walls (30 × 5 × 0.25 cm). Rats were individually placed on the central platform of the EPM facing an open arm in a 5-min test period. The entrance to a maze arm was defined if the rat places all four paws onto the arm. Rats were evaluated for time spent in the open arm and the number of entries into open arm as a measure of anxiety-like behavior. The number of open arm entries was calculated as the percentage of entries in the open arms = number of open arm entries/(number of open arm entries + number of closed arm entries) × 100. The time spent in the open arm was also evaluated as the percentage of time spent in open arms = time spent in the open arms/(time spent in open arms + time spent in the closed arms) × 100.

Western blot

To examine alterations in α_{1A}-AR and βArr2 protein levels in the hippocampus and PFC between groups, adolescent (PND 36; after the behavioral tests) and adult (PND 62) male rats ($N=8$ for each group) were used for western blot analysis. The animals were sacrificed by cervical dislocation, then the hippocampus and the PFC tissues were removed. The tissues were homogenized in ice-cold lysis buffer containing 50 mmol/L Tris, pH 8.0, 150 mmol/L NaCl, 1 mmol/L EGTA, 50 mmol/L NaF, 1.5 mmol/L MgCl2, 10% v/v glycerol, 1% v/v Triton X-100, 1 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L Na3VO4, and Complete Protease Inhibitor cocktail (Roche Diagnostics, Indianapolis, IN, USA). After centrifugation at 12,000 cycle/min for 10 min, the protein levels in the supernatants were determined and equal amounts of proteins were then loaded

onto a 10% polyacrylamide gel. After electrophoresis, the gels were transferred to polyvinylidene difluoride membranes. The membranes were blocked with 5% skim milk prepared in Tris-buffered saline with Tween (TBST) and incubated with primary antibodies α_{1A}-AR (Santa Cruz Biotechnology, Inc.), βArr2 (Santa Cruz Biotechnology, Inc.) and β-actin (Sigma-Aldrich) at 4 °C, overnight, and subsequently with the appropriate HRP-conjugated secondary antibodies for 1 h at room temperature and then visualized via enhanced chemiluminescence detection on the X-ray films. The result of the western blotting was scanned, and densitometric analysis for the quantification of the bands was done using Image J software (National Institutes of Health, Bethesda, MD, USA). Anti βarr2 and anti α_{1A}-AR were probed on the same gel. The α_{1A}-AR and βArr2 levels were normalized with that of β-actin, used for internal control protein.

Prazosin treatment

For α_{1A}-AR blockade, prazosin hydrochloride (Sigma) was intraperitoneally administered to adolescent rats at dosages of 0.2 and 1 mg/kg, 30 min before anxiety behavioral tests. In chronic injections, the same doses of prazosin were used for 5 consecutive days from PND 31 to PND 35. Rats were then tested at PND 35–36 for anxiety-like behavioral tests. Normal saline was administered to each control group. The doses of prazosin were chosen based on previous studies (Doze et al. 2009; Do-Monte et al. 2010; Funk et al. 2019).

Statistical analysis

Statistical analysis was performed using GraphPad Prism (GraphPad Software, San Diego, CA). Comparisons between two and three groups were analyzed by unpaired student's *t* test and one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test, respectively. In addition, differences within groups were analyzed by paired student's *t* test. Repeated measures ANOVA was used to determine the effect of MS on rat protein levels over time. The Pearson correlation coefficient was conducted to assess the correlation between animals' behavioral test performance and the protein expression in the PFC and hippocampus. All values are mean ± S.E.M. $p < 0.05$ was considered significant.

Results

Maternal separation induces anxiety-like behavior in adolescent rats

Increased anxiety in the EPM is related to a less preference for open arms. MS adolescent rats exhibited a significant reduction in time spent in the open arms ($p = 0.0036$) compared to controls (Fig. 1a), reflecting less time exploring open arms at stress group. We also found a significant decrease in the percentage of entries into open arm of stress group ($p = 0.0450$) compared to control group (Fig. 1b). These results suggest that MS increases anxiety-like behavior in adolescent rats. ($N = 8$ for each group).

Anxiety-like behaviors in the OF are related to reduced general locomotor activity and decreased time spent and distance traveled in the central area. In the present study, adolescent rats in stress group exhibited a significant decrease in time spent ($p = 0.0043$) and in the percentage of distance traveled ($p = 0.0013$) in the OF central area compared to control group (Fig. 1c, d). We also measured total distance traveled in the entire OF (as a measure of locomotor activity) and observed a significant reduction in stress group ($p = 0.013$) compared to controls (Fig. 1e). ($N = 8$ for each group).

Maternal separation differentially affects α_{1A}-AR and βArr2 expression in adolescent and adult male rats

The results of western blot revealed a significant decrease in α_{1A}-AR expression and a significant increase in βArr2 levels in both the hippocampus (for α_{1A}-AR: $p < 0.0001$, Fig. 2a; for βArr2: $p = 0.0005$, Fig. 2b) and PFC (for α_{1A}-AR: $p = 0.0086$, Fig. 3a; for βArr2: $p = 0.0001$, Fig. 3b) of MS adolescent rats compared to controls. ($N = 8$ for each group).

Repeated MS also led to changes in the α_{1A}-AR and βArr2 expression as measured in the hippocampus and PFC of 62-day-old rats. In contrast to adolescence, MS resulted in a significant increase in α_{1A}-AR expression ($p = 0.0213$) in the hippocampus of MS adult rats compared to controls (Fig. 2c), while changes in the hippocampal βArr2 levels were not significant ($p = 0.389$) (Fig. 2d). In the PFC of adult male rats subjected to MS, there was lower expression of α_{1A}-AR ($p = 0.0379$) and greater expression of βArr2 ($p = 0.0012$) than controls (Fig. 3c, d). These differences in the α_{1A}-AR and βArr2 expression between adolescent and adult male rats suggest that MS differentially influences α_{1A}-AR and βArr2 levels during adolescence and adulthood. ($N = 8$ for each group).

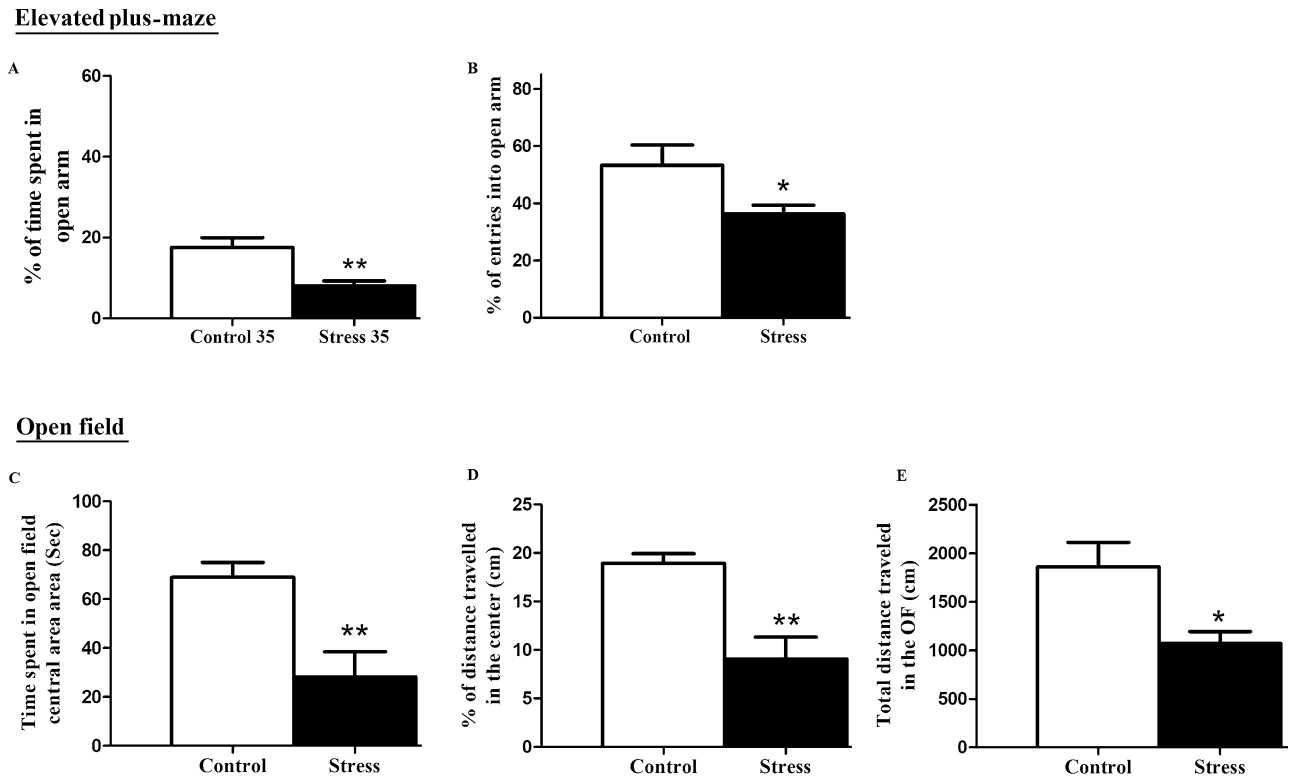

Fig. 1 Effects of MS on anxiety-like behaviors in adolescent rats in the EPM and OF. **a**, **b** The percentage of open arm time and open arm entries in the EPM were markedly decreased in stress group (For % of open arm entries $p=0.0450$; for % of open arm time $p=0.0036$) compared to controls. **c** In the OF, MS adolescent rats spent significantly less time in the central area ($p=0.0043$). **d** Distance traveled in the OF central area was significantly reduced in stress group ($p=0.0013$) compared to controls. **e** Total distance traveled in the OF (an index of locomotor activity) was significantly reduced in stress group ($p=0.013$) compared to controls. The results are shown as mean + SEM ($N=8$ for each group). *$p<0.05$, **$p<0.01$ significant difference compared to control group (unpaired *t* test)

The comparison of developmental effects of MS on the protein levels indicated that changes in the expression of α_{1A}-AR and βArr2 of MS adolescent rats were reversed in MS adult rats, with a significant increase in the expression of α_{1A}-AR and a marked decrease in βArr2 expression in both the hippocampus (for α_{1A}-AR: $p=0.0001$, Fig. 2e; for βArr2: $p=0.0012$, Fig. 2f) and PFC (for α_{1A}-AR: $p=0.0467$, Fig. 3e; for βArr2: $p=0.0264$, Fig. 3f) of MS adult rats compared with MS adolescence. ($N=8$ for each group).

Anxiety-like behavior is inversely correlated with α_{1A}-AR expression

As shown in Fig. 4, there is a positive association between time spent in the OF central area (as inverse index of anxiety-like behavior) with α_{1A}-AR levels in both hippocampus (control group: $r=0.80$, $p=0.0159$; stress group: $r=0.90$, $p<0.01$; Fig. 4a) and PFC (control group: $r=0.83$, $p<0.01$; stress group: $r=0.90$, $p<0.01$; Fig. 4b).

Acute and chronic treatment with prazosin significantly increased anxiety-like behaviors in adolescent male rats

Since an anxiety-like phenotype in MS adolescent rats was associated with α_{1A}-ARs downregulation, we hypothesized that this behavior could be mimicked by treating developing rats with an α_{1A}-AR antagonist. We used prazosin, an α_{1A}-AR antagonist (Doze et al. 2009), to determine whether the anxiety-like phenotype could be induced in adolescent rats by blocking α_{1A}-AR. Intraperitoneal injection of prazosin at dosages of 0.2 mg/kg and 1 mg/kg (Doze et al. 2009; Do-Monte et al. 2010; Funk et al. 2019), was performed 30 min prior to anxiety behavioral tests. We also investigated the effects of chronic prazosin treatment of developing rats on anxiety-like behaviors in adolescent rats. Prazosin at dosages of 0.2 mg/kg and 1 mg/kg was administered to rats for 5 consecutive days from PND 31 to PND 35. Rats were then tested for anxiety behavioral tests at PND 35–36.

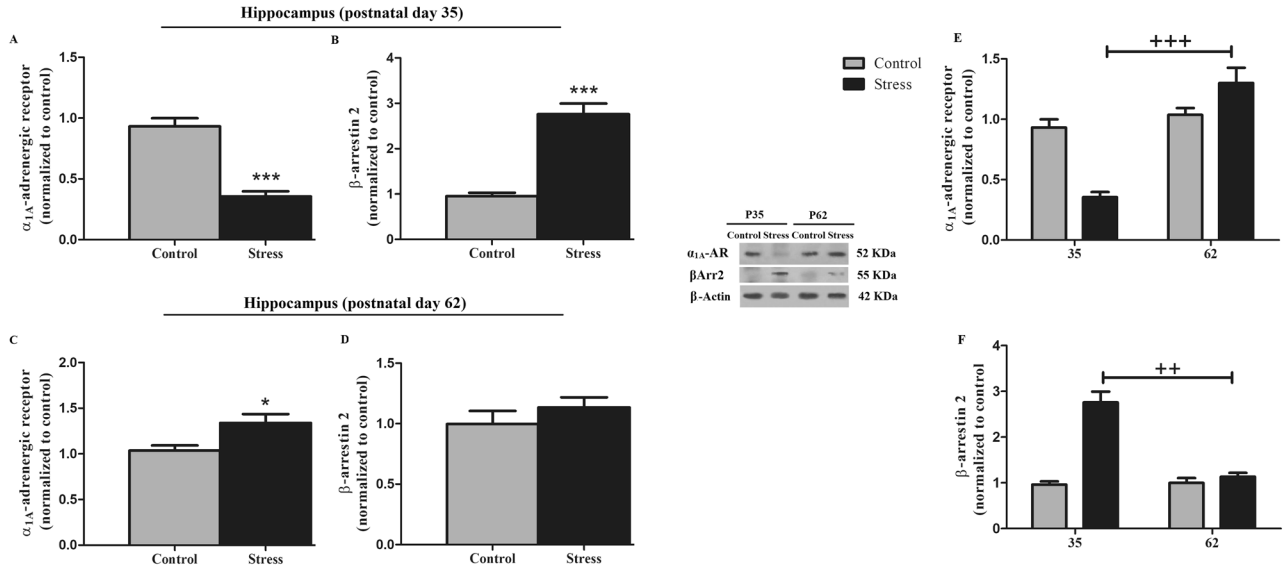

Fig. 2 Changes in α_{1A}-AR and βArr2 levels in the hippocampus of adolescent and adult male rats subjected to MS. **a**, **b** In adolescent rats, MS significantly decreased α_{1A}-AR expression ($p<0.0001$), while markedly increased βArr2 levels ($p=0.0005$) in the hippocampus compared to control group. **c**, **d** In adult rats, MS significantly increased the α_{1A}-AR levels ($p=0.0213$), while did not significantly affect βArr2 levels ($p=0.389$) in the hippocampus compared to controls. **e**, **f** The comparison of protein expression within stress groups at PND 35 and PND 62 indicated a significant increase in the expression of α_{1A}-AR ($p=0.0001$, Fig. 2e) and a marked decrease in βArr2 expression ($p=0.0012$, Fig. 2f) in the hippocampus of stress adults compared to stress adolescent rats. The results are shown as mean + SEM ($N=8$ for each group). $^{*}p<0.05$, $^{***}p<0.001$ significant difference compared to control group (unpaired t test). $^{+}p<0.01$, $^{+++}p<0.001$ significant difference compared to stress group (paired *t* test)

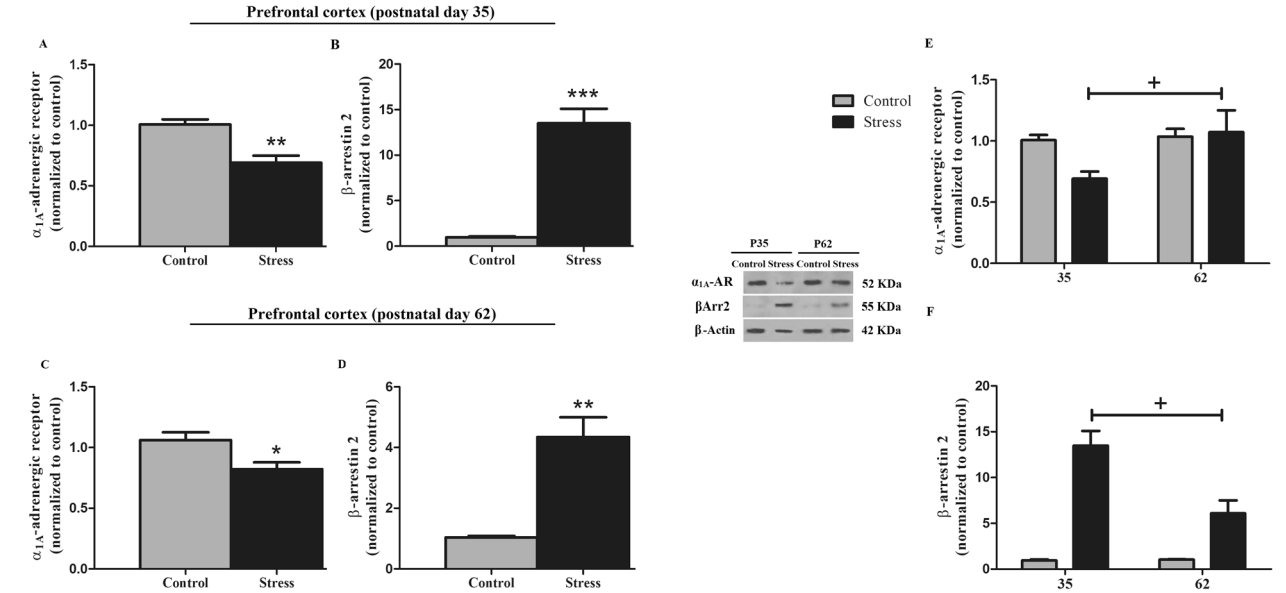

Fig. 3 Changes in α_{1A}-AR and βArr2 levels in the PFC of adolescent and adult male rats subjected to MS. **a**, **b** In adolescent rats, MS significantly reduced the α_{1A}-AR expression ($p=0.0086$), while increased βArr2 levels ($p=0.0001$) in the PFC compared to control group. **c**, **d** In adult rats, MS significantly decreased the α_{1A}-AR levels ($p=0.0379$), while increased βArr2 levels ($p=0.0012$) in the PFC compared to control group. **e**, **f** The comparison of protein expression within stress groups at PND 35 and PND 62 indicated a marked increase in the expression of α_{1A}-AR ($p=0.0467$, Fig. 3e) and a significant decrease in βArr2 expression ($p=0.0264$, Fig. 3f) in the PFC of stress adults compared to stress adolescent rats. The results are shown as mean + SEM ($N=8$ for each group). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significant difference compared to control group (unpaired *t* test). $^{+}p<0.01$ significant difference compared to stress group (paired *t* test)

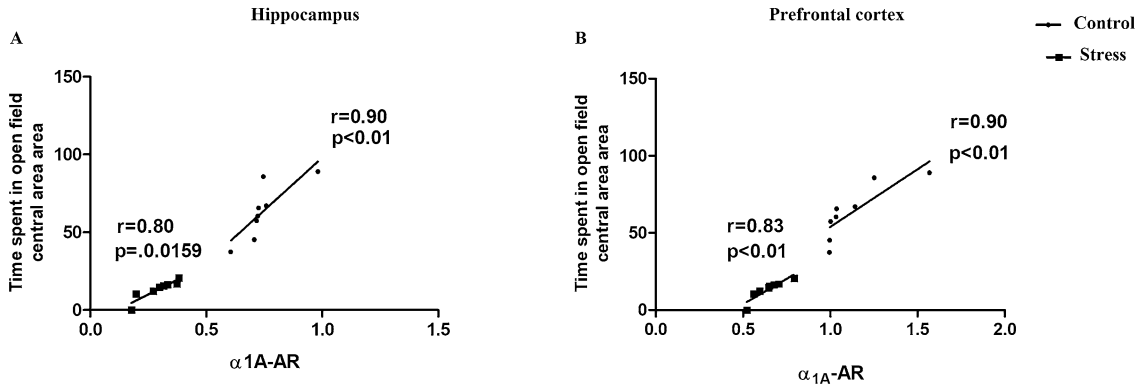

Fig. 4 Anxiety-like behavior is inversely correlated with α_{1A}-AR levels. **a** A positive association between time spent in the open field central area and α_{1A}-AR levels in the hippocampus (control group: $r=0.80$, $p=0.0159$; stress group: $r=0.90$, $p<0.01$). **b** Direct association between time spent in the open field central area and α_{1A}-AR levels in the PFC (control group: $r=0.83$, $p<0.01$; stress group: $r=0.90$, $p<0.01$). The results are shown as mean + SEM ($N=8$ for each group)

In the OF, a single acute injection of prazosin at dosages of 0.2 and 1 mg/kg during PND 35 resulted in a significant less time spent [$F(2,18)=9.556$, $p=0.0015$; Fig. 5a] and lower distance traveled [$F(2,17)=18.83$, $p<0.0001$; Fig. 5b] in the center in adolescent rats than controls, suggesting a higher level of anxiety in acute prazosin group than control group. Similar results were obtained with chronic administration of prazosin (at dosages of 0.2 and 1 mg/kg) [for time spent in the center: $F(2,18)=7.511$, $p=0.0042$, Fig. 5d; for distance traveled in the center: $F(2,17)=6.122$, $p=0.0099$, Fig. 5e]. Furthermore, adolescent rats in both acute [$F(2,17)=17.29$, $p<0.0001$] and chronic [$F(2,18)=11.68$, $p=0.0006$] groups displayed hypoactivity, shown by reduced total distance traveled in the OF compared to controls, consistent with an increase in anxiety levels (Fig. 5c, f).

In the EPM, adolescent rats with acute and chronic injection of prazosin spent less time in open arm [for acute prazosin injection: $F(2,18)=16.79$, $p<0.0001$, Fig. 5g; for chronic prazosin injection: $F(2,18)=5.648$, $p=0.0125$,

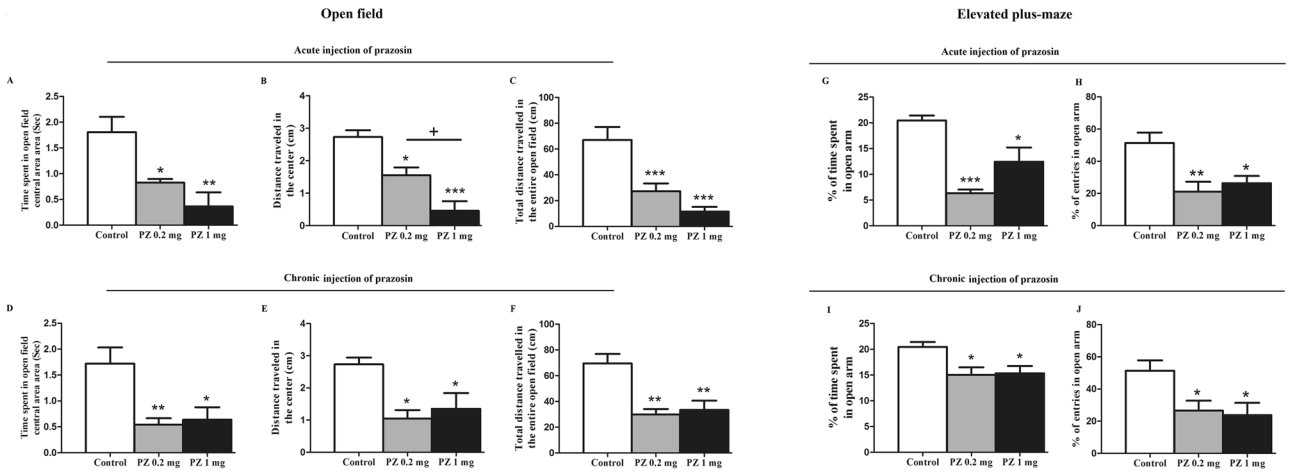

Fig. 5 Acute and chronic prazosin administration during development results in anxiety-like behaviors in adolescent rats in the EPM and OF. In the OF, time spent in the central area (for acute group: $p=0.03$; for chronic group: $p=0.005$) (panel **a**, **d**), distance traveled in the center (for acute group: $p=0.0036$; for chronic group: $p=0.0428$) (panel **b**, **e**) and total distance traveled in the OF (for acute group: $p=0.0031$; for chronic group: $p=0.0063$) (panel **c**, **f**) were significantly decreased following prazosin injection in both acute and chronic injection groups compared to controls. In the EPM, the percentage of time spent in open arm (for acute group: $p=0.0269$; for chronic group: $p=0.0214$) (panel **g**, **i**) and open arm entries (for acute group: $p=$ for chronic group: $p=$) (panel **h**, **j**) were markedly decreased in both acute and chronic groups compared to controls. The results are shown as mean + SEM ($N=7$ for each group). $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ significant difference compared to control group (One way ANOVA). $^{+}p<0.01$ significant difference compared to prazosin group

Fig. 5i] and had lower number of open arm entries [for acute prazosin injection: $F(2,18) = 7.858$, $p = 0.0035$, Fig. 5h; for chronic prazosin injection: $F(2,17) = 5.175$, $p = 0.0176$, Fig. 5j] than controls. ($N = 7$ for each group).

Taken together, these data provide further evidence that MS-induced anxiety-like behavior in adolescent rats may in part be due to decreased α_{1A}-AR signaling.

Discussion

Early-life stress is known to interfere with brain development and maturation, increasing later risk for a variety of psychiatric disorders, including anxiety disorders, in part via perturbing the programming of the limbic system including hippocampus and prefrontal cortex, two essential mediators of early-life stress on subsequent behavior later in life (Teicher et al. 2003; Champagne et al. 2008; Lupien et al. 2009; Kim et al. 2015; Arnsten et al. 2015). Adolescence is a crucial period of development that is vulnerable to the onset of specific mental health problems (Kessler et al. 2012). Since βArrs are assumed to be involved in the regulation of GPCRs including ARs, hence, this study was conducted to examine few consequences of repeated early-life stress by evaluating the effects of early MS on changes in α_{1A}-AR and βArr2 expression in the PFC and hippocampus of adolescent and adult male rats. We also investigated the presence of a possible relationship between the protein levels and anxiety behavior in adolescent male rats as well as the effect of reducing α_{1A}-AR neurotransmission during development on anxiety-like responses in adolescent animals.

Assessment of anxiety behavior of adolescent rats in the EPM revealed a higher level of anxiety in stress group, shown by lower percentage of time spent in the open arm and open arm entries. The OF data indicated decreased time spent and distance traveled in the center (as indicators of anxiety-like behavior) in MS adolescent rats, which indicates more anxiety than control rats. In addition, the percentage of total distance traveled in the center (as an indicator of locomotor activity) was decreased in MS adolescent rats. In our study, developing brain also responded to repeated MS by downregulating α_{1A}-ARs in the PFC and hippocampus. This MS-induced decrease in α_{1A}-AR expression in the hippocampus and PFC of adolescent rats was reversed in adult rats, with a significant increase in the α_{1A}-AR levels in both brain regions, suggesting that MS differentially influences α_{1A}-AR expression during adolescence and adulthood. Previous research has shown conflicting results for the effect of early-life stress on anxiety as well as on α_1-AR expression in developing and mature brain, likely in part due to different stress protocols used. For example, prenatal stress was found to cause a reduction in α_1-AR binding in cerebral cortex of rats at 16 but not at 23, 40 or 60 days of age (Peters 1984). In another study, MS (from days 1–13) produced enduring downregulation of α_1-ARs in the prefrontal-limbic forebrain/limbic midbrain network in adult mice (Coccurello et al. 2014). Regarding the effects of MS on anxiety behavior, Jin et al. reported that MS (3 h from PND1-21) produced a significant decrease in locomotor activity and increased anxiety-like behaviors in adolescent rats (Jin et al. 2018), while MS for 4 h per day from PND1-21 resulted in enhanced locomotor activity and reduced anxiety behavior of adolescent rats in the OF (Qiong et al. 2015).

Novelty-seeking has been shown to affect anxiety-like behavior in the OF (Prut and Belzung 2003), and exploratory behavior in response to novelty appears to be largely mediated via activation of noradrenergic system (Sara et al. 1995; Rebec et al. 1997; Stone et al. 1999, 2006, 2011; Collier et al. 2004). Mc Fie et al. indicated that clozapine, which has a strong α_1-AR antagonistic effect, reduces exploratory activity and increases anxiety-like behavior in Wistar-Kyoto rats at PND 35–36 (Mc Fie et al. 2012). This is consistent with our findings that revealed that a decreased level of α_{1A}-AR in MS adolescent rats strongly correlates with an increase in anxiety-like behavior in the animals. Other studies have also demonstrated that α_1-AR antagonists reduce locomotor activity in several rodent models of hyperactivity (Velley et al. 1982; Snoddy and Tessel 1985; Blanc et al. 2002), supported by the studies that demonstrated Wistar-Kyoto rats, which have hypofunctionality of noradrenergic neurotransmission (Howells and Russell 2008; Howells et al. 2012), are hypoactive compared to other species rats (Gentsch et al. 1987; Pare 1994; Ferguson and Cada 2003). These data and the result obtained from our study suggest a role of α_{1A}-AR in exploratory behavior in response to novelty.

Downregulation of α_1-ARs is a mechanism of AR deactivation (Finch et al. 2006) and occurs via receptor internalization, enhanced degradation via endosomal-lysosomal system, or decreased the reporter mRNA expression (Lefkowitz 1998). βArrs could interact to α_1-ARs and potentially cause functional alterations (Uberti et al. 2003). In the present study, in contrast to α_{1A}-AR, MS resulted in increased expression of βArr2 in the PFC and hippocampus of adolescent rats, suggesting the existence of a mechanism of adrenergic deactivation likely in response to stress-induced increased βArr2 levels. However, it has been unclear whether downregulation of α_{1A}-AR expression during development may be involved in modulation of anxiety-like behaviors in adolescent rats. In the present study, we indicated that acute and chronic pharmacological blockade of α_{1A}-AR with prazosin, a α_{1A}-AR antagonist, resulted in increased anxiety-like behavior and decreased activity in adolescent rats, suggesting that MS-induced anxiety-like behavior in adolescent rats may in part due

to decreased α_{1A}-AR signaling. In our study, MS-induced increase in βArr2 expression in the hippocampus and PFC of adolescent rats was reversed in adult rats. This in turn may affect BArr2 in adolescent and adult rats. βArrs have been linked to anxiety regulation (Asth et al. 2016; Ding et al. 2017; Robins et al. 2018). For example, mice lacking βArr1 in mice decreased anxiety in both sexes (Robins et al. 2018). Furthermore, nociceptin/orphanin FQ (N/OFQ) receptor agonists can cause anxiolytic-like effect in rodents and this effect has been shown to be determined by βArr2 recruitment (Asth et al. 2016). These data suggest that developing and mature brains are differentially regulated by βArr2.

Differences observed in the α_{1A}-AR expression and βArr2 (as a possible regulator of α_{1A}-AR expression) in developing and adult brain following repeated MS exposure may have long-term effects on α_{1A}-AR function, as shown for α_2-AR. For example, a differential response of the developing and mature brain to norepinephrine regulation of α_2-AR density has been demonstrated in previous research (Sanders et al. 2011), and has been suggested to be in part responsible for the therapeutic effects of tricyclic antidepressants in adult depression and the lack of their efficacy in the treatment of childhood depression (Deupree et al. 2007; Bylund and Reed 2007; Sanders et al. 2011).

Together, our findings suggest a possible interaction of βarr2 with α_{1A}-AR in the PFC and hippocampus of adolescent rats. Early-life stress increase in βArr2 levels may promote this interaction and decrease α_{1A}-AR neurotransmission via downregulating the receptors, which in turn could facilitate anxiety-like behavior. Hence, targeting βArr2 may provide a new opportunity for developing more effective anxiolytic treatments.

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Compliance with ethical standards

Conflict of interest The authors have declared no conflict of interest.

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