Neurosci Bull October 1, 2013, 29(5): 531–540. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1350-y

·Original Article·

β-Arrestin-2 inhibits preference for alcohol in mice and suppresses Akt signaling in the dorsal striatum

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ABSTRACT

In this study, we investigated the role of β-arrestin-2 in alcohol preference using the two-bottle choice and conditioned place preference procedures in wild-type (WT) and β-arrestin-2 knockout (KO) mice. Locomotion and righting reflex tests were performed to test alcohol sensitivity. The possible molecular signals regulated by β-arrestin-2 were analyzed by Western blot. We found that β-arrestin-2 KO mice showed a marked increase in voluntary alcohol consumption without significant differences in preference for saccharin or aversion to quinine. These animals also exhibited higher conditioned place preference scores for alcohol than WT mice. Meanwhile, KO mice showed reduced sensitivity to alcohol and increased blood alcohol clearance. Furthermore, after the free consumption of alcohol, the activities of protein kinase B and glycogen synthase kinase 3β (GSK3β) increased in the dorsal striatum of WT mice, but not in KO mice, which showed high basal activity of Akt in the dorsal striatum. These results suggest that β-arrestin-2 negatively regulates alcohol preference and reward, likely through regulating the activation of signaling pathways including Akt/GSK3β in the dorsal striatum.

Keywords: β-arrestin; alcohol preference; Akt

INTRODUCTION

Alcohol addiction is a complex behavioral disorder characterized by repetitive and excessive alcohol drinking, which ultimately leads to a loss of control over consumption, the development of tolerance and dependence, and impairment of social and occupational functions[1]. Studies indicate that the development of alcohol addiction is a result of the interplay between genetics and social environment[2,3]. Accumulating evidence has suggested that neuronal responses to alcohol involve several G protein-coupled receptor (GPCR)-mediated signaling pathways, inducing short-term to long-term changes in behavioral and neuronal plasticity[4]. Besides, alcohol increases dopamine release into the nucleus accumbens from projections that originate in the ventral tegmental area[5], and dopamine D2 receptors in the nucleus accumbens regulate alcohol preference and intake[6]. Furthermore, an antagonist selective against dopamine D3 receptors decreases alcohol intake and prevents the relapse to alcohol-seeking behavior[7]. The G-protein βγ dimers regulate alcohol consumption through a synergy of D2 and adenosine A2 receptor-stimulated PKA signaling[8]. It was reported that agonists of the adenosine A2A receptor reduce alcohol consumption in alcohol-preferring rats[9]. Other GPCRs are also involved in alcohol dependence. Antagonists of CB1 cannabinoid and mGluR5 metabotropic receptors reduce alcohol intake in several animal models[10,11]. These studies indicate that neurons in the limbic system orchestrate different GPCR signals during alcohol consumption; however, the underlying mechanisms are not clear.

β-Arrestin-1 and β-arrestin-2 are important members of the arrestin family, and are ubiquitously expressed

in the central nervous system in newborn and adult animals[12,13]. Previous studies have shown that β-arrestins attenuate GPCR-mediated signal transduction and induce receptor internalization in an agonist-dependent manner[14]. β-Arrestins also interact with many signaling molecules and serve as scaffold proteins[15]. However their specific functions in the nervous system are not fully understood, though studies suggest that they are involved in the development of substance dependence. Our earlier study showed that chronic morphine treatment and withdrawal regulate β-arrestin expression in the limbic system[16]. Bohn and colleagues reported that β-arrestin-2 knockout (KO) mice exhibit enhanced morphine analgesia, reduced tolerance to morphine, and enhanced morphine-conditioned place preference[17-20]. Recent studies showed that β-arrestins function as signaling molecules in the nervous system[21]. For example, β-arrestin-2 forms a signal complex with Akt to mediate dopamine-dependent behaviors[22], interacts with ERK and affects its kinase activity, and is responsible for morphine-dependent D1 receptor activation and increased locomotion[23]. Moreover, Bjork *et al.* reported that β-arrestin-2 deficient mice exhibit reduced voluntary consumption of a solution containing 6% alcohol and 0.2% saccharin[24], suggesting that it plays a role in alcohol dependence. This study therefore aimed to investigate the role of β-arrestin-2 and the possible molecular signals in alcohol preference.

MATERIALS AND METHODS

Animals

β-Arrestin-2 KO and β-arrestin-1 KO mice generated in the laboratory of R. J. Lefkowitz (Duke University Medical Center, Durham, NC) were back-crossed to C57BL/6J for 10 generations. Wild-type (WT) or β-arrestin KO littermates from heterozygous and descendants resulting from homozygous breeding of WT or β-arrestin KO mice after the initial back-crossing were used. All mice were maintained on a 12:12 h light/dark reverse daylight cycle with food and water available *ad libitum*. All experiments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by Animal Care and Use Committee of Shanghai Medical College of Fudan University. In all tasks, 2–3-month-old male mutant and control littermates were used.

Two-Bottle Choice Model

To measure alcohol preference, a two-bottle free-choice procedure was performed in WT, β-arrestin-1 KO, and β-arrestin-2 KO mice. One bottle was filled with sterilized tap water and the other contained different concentrations of alcohol (3% to 15%, *v/v*)[25]. Animals were single-housed and had continuous access to the contents of both bottles. They were exposed to ascending alcohol concentrations for 4–5 days. Alcohol and water intake were measured daily at 13:00, and the bottle position was switched daily to avoid side preference. One month after this procedure, the saccharin (sweet) and quinine (bitter) intake and preference tests were performed in these mice as previously described[26]. Mice were sequentially given two concentrations of saccharin (0.03% and 0.06%) and quinine (15 and 30 μmol/L), *versus* tap water (tap water in one bottle and saccharin or quinine in the other), with free access to each solution for two days. The bottle positions were also switched daily. Water and saccharin or quinine intake were also measured daily at 13:00. Data were analyzed by two-way ANOVA. The amount of alcohol consumed each day was normalized to the body weight as g/kg per day. The alcohol preference score was calculated as the percentage of alcohol in the total volume of fluid consumed. The amount of saccharin and quinine consumed was expressed as mL/day.

Locomotion Test

Plexiglas chambers (43 cm^2) were used for the locomotion test (Med-Associates, St. Albans, VT), and basal activity was recorded for 60 min once a day for three days. Basal activity was expressed as the distance traveled in the chamber (cm/h). On the fourth day, WT and β-arrestin-2 KO mice were given one injection of 20% alcohol (2.0 g/kg, i.p.) immediately before the start of activity monitoring. Horizontal traveled distance (cm) was recorded for 15 min after alcohol injection[27].

Righting Reflex Tests

Mice were given an injection of a high dose of 20% alcohol (4.0 g/kg, i.p.) and were intermittently placed on their backs. The time (min) to regain the righting reflex was measured

as described previously[28].

Alcohol-Induced Conditioned Place Preference (CPP)

Alcohol-induced CPP was measured in a two-chamber apparatus (each chamber was 27.3 × 27.3 cm^2, Med Associates) with different floor textures (rods or a grid) and a central partition with a manual door. The protocol for alcohol-induced CPP behavior was adopted from previous studies[29,30]. Before the conditioning training phase, a habituation test was done on day 1. In this test, mice were placed in the CPP apparatus and allowed free access to the entire apparatus for 15 min. The time spent in each chamber was recorded to detect any initial bias. Mice that showed a basal preference >65% in either chamber were excluded from the study. Conditioning training consisted of two conditioning trials per day for three days (days 2–4). On the morning of day 2, mice were given saline solution (10 mL/kg; i.p.) and confined immediately to one of the compartments for 5 min (unpaired compartment). In the afternoon of the same day, mice were given alcohol (2.0 g/kg, i.p.) and confined to the other compartment (drug-paired compartment). This schedule was repeated on days 3 and 4. On day 5, the mice were allowed to explore the entire apparatus for 15 min (post-conditioning test). The CPP score was calculated as the time spent in the alcohol-paired compartment on the test day minus the time spent in the same compartment on the preconditioning day.

Alcohol Clearance

Blood alcohol concentration (BAC) was measured after a single injection of 20% alcohol (3.5 g/kg, i.p.). Blood was collected into a sterile microcentrifuge tube by angular vein puncture. Plasma was collected and frozen at −20°C until analysis. Blood samples were obtained at 15, 30, 60, 90, 150, and 180 min after injection. BAC was determined by the NAD-ADH enzymatic assay (Sigma-Aldrich, St. Louis, MO).

Western Blotting

After one-month stable voluntary consumption of 9% alcohol, mouse brains were collected, the dorsal and ventral striatal areas dissected out and homogenized, and protein concentrations in the tissue lysates were determined by the Bradford method. Samples diluted in sample buffer (0.3 mmol/L Tris-HCl, pH 6.8, 30% glycerol, 10% SDS, 6% β-mercaptoethanol, and 0.012% bromophenol blue) were heated at 95°C for 5 min, and a 30-μg aliquot was loaded onto 10% SDS polyacrylamide gel. After electrophoresis, proteins were transferred onto nitrocellulose membranes. The membranes were washed in TBST and incubated with primary antibodies overnight followed by IRDye® 700DX-conjugated anti-rabbit IgG (Rockland Immunochemicals, Gilbertsville, PA, 1:50 000; emission wavelength, 700 nm) for 1 h before scanning. The protein levels of Akt, glycogen synthase kinase 3β (GSK3β), ERK, and CREB were also analyzed with each corresponding antibody. The antibodies against pAkt (Thr-308, Thr-473), pGSK3β (Ser-9), pERK, and pCREB were from Cell Signaling Technology (Boston, MA; 1:1 000). The levels of phosphorylation of Akt, GSK3β, ERK, and CREB were normalized to the saline-treated WT group.

Data Analysis

Comparisons of genotype × alcohol concentration effects on behaviors between groups were performed using two-way ANOVA followed by the *post hoc* Tukey test. BAC data were analyzed by *t*-test at each time point. CPP and Western blot data were evaluated using one-way or two-way ANOVA followed by the *post hoc* Tukey's test. Comparisons between two groups were performed with the *t*-test. Data are presented as mean ± SEM. Statistical significance was set at P <0.05.

RESULTS

β-Arrestin-2 Deficiency Enhanced Alcohol Consumption

To test whether β-arrestin-2 is necessary for alcohol consumption and preference, the β-arrestin-2 KO mice underwent the two-bottle choice test for alcohol preference. At lower alcohol concentrations (3% to 7%), both WT and KO mice displayed increased alcohol intake as the concentration increased, and reached comparable levels of alcohol consumption; at high alcohol concentrations (9% to 15%), the KO mice consumed a progressively greater amount of alcohol than WT mice [$F_{genotype \times concentration}$ (6, 230) = 5.805, P <0.001; Fig. 1A]. KO mice also showed a greater preference for alcohol than WT mice at high concentrations (9% to 15%), but showed no significant interaction between genotype and alcohol concentration [$F_{concentration}$ (6, 230) = 8.004, P <0.001; $F_{genotype}$ (1, 230) = 20.663, P <0.001; $F_{genotype \times concentration}$ (6, 230) = 0.670, P = 0.674; Fig. 1B].

To exclude the possibility that the increased preference for alcohol was due to an abnormal taste sensitivity caused by the lack of β-arrestin-2, the mice were tested for their consumption of bitter (quinine) and sweet (saccharin) solutions, and there was no difference in preference for saccharin or aversion for quinine between WT and KO mice [$F_{genotype}$ (3, 41) = 2.793, P = 0.103; Fig. 1C]. These results indicate that the changes in alcohol intake and preference observed in KO mice are not attributable to impaired taste perception, and suggest that β-arrestin-2 negatively regulates alcohol intake in mice.

β-Arrestin-1 KO mice also underwent the two-bottle choice alcohol consumption task, and interestingly, these mice did not differ in alcohol intake and preference from their WT littermates (Fig. 2) [$F_{genotype \times concentration}$ (4, 59) = 0.100, P = 0.982 for alcohol intake; $F_{genotype \times concentration}$ (4, 59) = 0.130, P = 0.971 for alcohol preference]. These results indicate that β-arrestin-1 and β-arrestin-2 are differentially involved in alcohol-induced responses.

β-Arrestin-2 Deficiency Increased Alcohol-induced CPP

During the habituation phase of the CPP test, no side preference occurred in both genotypes (Fig. 3) [$F_{genotype}$ (1, 50) = 0.001, P = 0.997]. After three days of alcohol conditioning, both WT and β-arrestin-2 KO mice showed a significant preference for the alcohol-paired chamber, but the KO mice had higher CPP scores than their WT littermates [$F_{genotype \times treatment}$ (1, 74) = 4.710, P = 0.033; Fig. 3], indicating the negative regulation of alcohol reward and preference by β-arrestin-2.

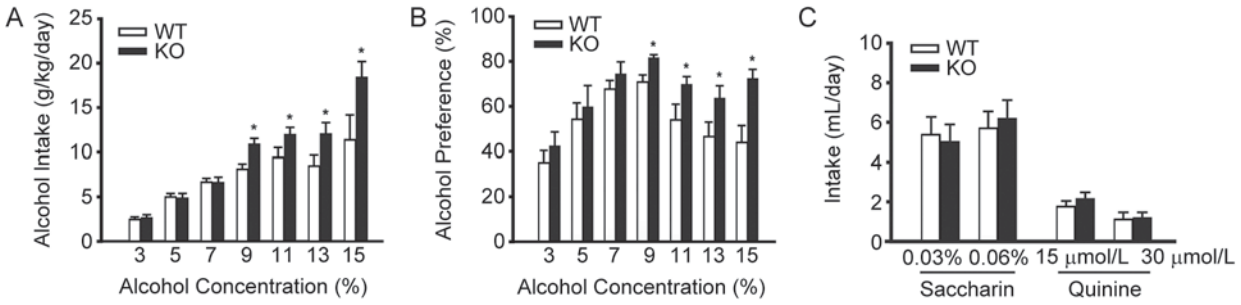

Fig. 1. β-Arrestin-2-deficiency enhanced alcohol consumption and preference. A and B: Mice underwent the alcohol two-bottle choice paradigm, and alcohol intake (A) and preference (B) were measured. *n* = 16–17 for each genotype. C: Saccharin preference and quinine aversion. *n* = 10 for each genotype. All values are presented as mean ± SEM. **P* <0.05 *versus* WT group; ANOVA and *post hoc* Tukey's test.

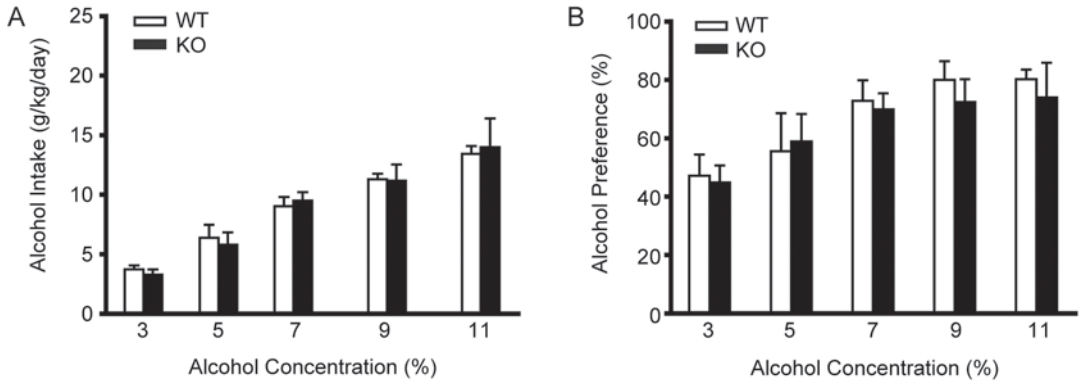

Fig. 2. β-Arrestin-1-deficiency did not change alcohol consumption (A) and preference (B) in mice. β-Arrestin-1 knockout (KO) mice underwent the alcohol two-bottle choice paradigm. *n* = 6–7 for each genotype. Data are presented as mean ± SEM. Two-way ANOVA and *post hoc* Tukey's test.

β-Arrestin-2 Deficiency Decreased the Response to Acute Alcohol Treatment

Numerous studies have demonstrated an inverse relationship between the motivation for alcohol consumption and alcohol sensitivity[25]. Therefore, the alcohol-induced locomotor activity and righting reflex tests were used to assess the alcohol sensitivity of the mice. Basal spontaneous activity and the locomotion stimulated by acute alcohol injection (2 g/kg i.p.) were tested. There was no significant difference in basal locomotion between WT and β-arrestin-2 KO mice [Fig. 4A left; $F_{genotype \times days}(2, 67) = 0.393$, $P = 0.676$]. During the 15 min immediately after an injection of alcohol (2.0 g/kg i.p.), WT mice showed higher locomotor activity than KO mice (Fig. 4A) [*t*-test, $P = 0.046$], indicating that KO mice have a reduced response or sensitivity to alcohol. Moreover, the time required for regaining the righting reflex after an injection of alcohol (4.0 g/kg) was markedly decreased in β-arrestin2 KO mice compared with WT littermates (Fig. 4B) [*t*-test, $P = 0.008$].

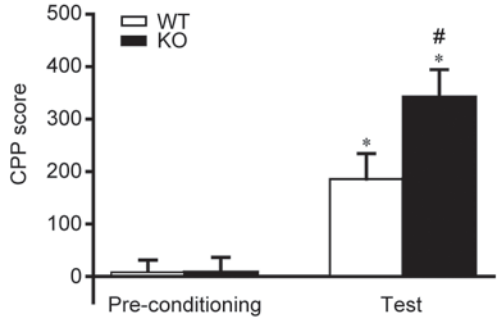

Fig. 3. β-Arrestin-2 deficiency increased alcohol-induced conditioned place preference (CPP). After three days of alcohol conditioning, WT and β-arrestin-2 knockout (KO) mice were tested for CPP. *P <0.05 *vs* corresponding pre-conditioning group; #P < 0.05 *vs* WT test group. n = 12–25/group. Data were analyzed by two-way ANOVA followed by the *post hoc* Tukey's test.

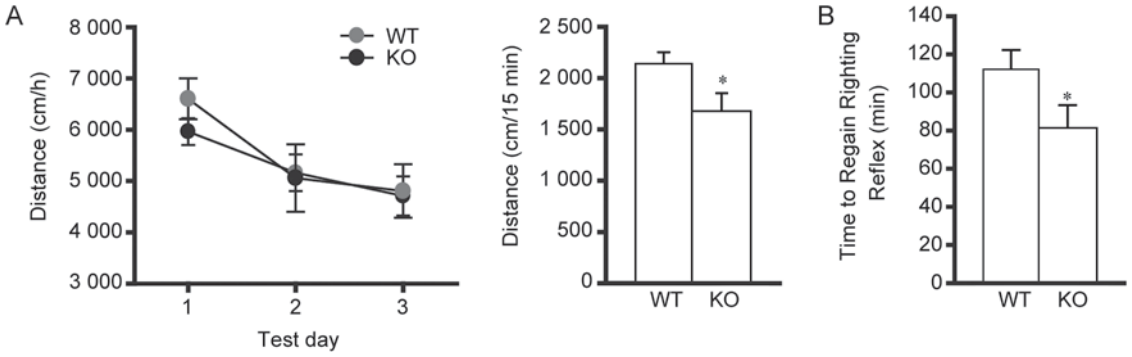

Fig. 4. β-Arrestin-2 deficiency impaired acute responses to alcohol. A: Left, basal activity of WT and β-arrestin-2 knockout (KO) mice on three days; right, locomotor activity of WT and KO mice after receiving an injection of 20% alcohol (2.0 g/kg, i.p.). B: Latency to regain the righting reflex. WT mice and KO mice received an injection of 20% alcohol (4.0 g/kg, i.p.). The mice needed a long time to be awake after alcohol injection. n = 11–13 for each group. Data are presented as mean ± SEM. *P <0.05, Student's *t*-test.

β-Arrestin-2 Deficiency Increased Blood Alcohol Clearance

β-Arrestin-2 KO mice had a lower BAC than their WT littermates at 60 and 90 min after alcohol injection (Fig. 5) [*t*-test, $P = 0.041$ for 60 min; $P = 0.005$ for 90 min]. No differences in clearance rates were detected at 15, 30, 150, or 180 min.

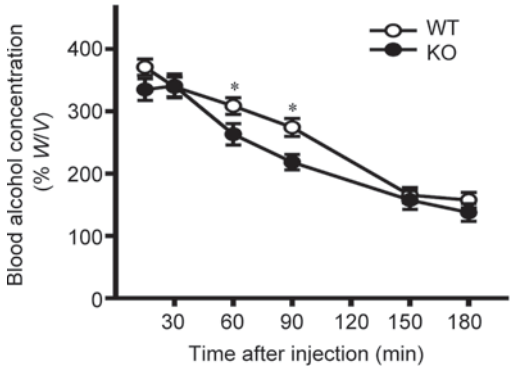

Fig. 5. Alcohol clearance in WT and β-arrestin-2 knockout (KO) mice. Blood alcohol levels were measured after a single injection of 20% alcohol (3.5 g/kg, i.p.). n = 19–22/group. Data are presented as mean ± SEM. *P <0.05 *vs* KO at the same time point, Student's *t*-test.

Ablation of β-Arrestin-2 Inhibited Phosphorylation of Akt/GSK3β in the Dorsal Striatum Induced by Chronic Alcohol Consumption

It has been shown that β-arrestin-2 forms a complex with Akt and protein phosphatase 2 (PP2A), and thus is involved in D2 receptor-mediated dopaminergic signaling through dephosphorylating Akt and in turn activating GSK3β[22]. We thus examined the effect of chronic alcohol consumption and ablation of β-arrestin-2 on the activity of Akt/GSK3β. After one month of voluntary consumption of

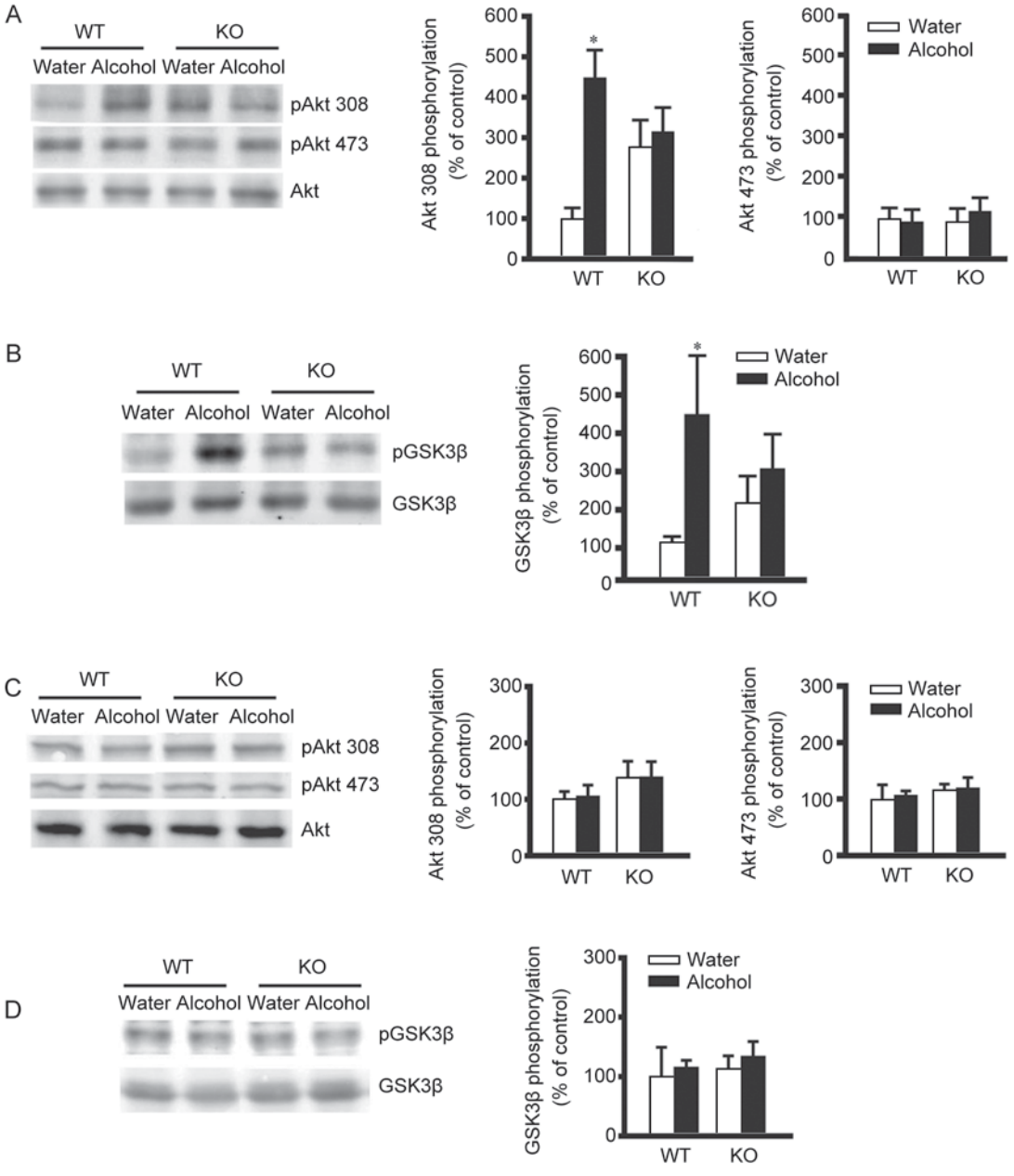

Fig. 6. β-Arrestin-2 was required for the regulation of Akt/GSK3β signaling in response to chronic alcohol consumption. After one month of voluntary consumption of 9% alcohol or tap water, pAkt (A, C) and pGSK3β (B, D) levels in the dorsal (A, B) and ventral striatum (C, D) from WT and β-arrestin-2 knockout (KO) mice were determined by Western blot. *n* = 3–6/group. All values are presented as mean ± SEM. **P* <0.05 *vs* WT/Water, ANOVA, *post hoc* Tukey's test.

9% alcohol, the pAkt levels in the dorsal striatum of WT and β-arrestin-2 KO mice were determined by Western blotting. The chronic intake of alcohol increased the pAkt level at Thr308 (pAkt 308) in WT mice (Fig. 6A) [$F_{treatment}$ (1, 12) = 21.785, P <0.001]. Meanwhile, a parallel increase of dorsal striatal pGSK3β, a downstream substrate of Akt, was also detected in WT mice (Fig. 6B) [$F_{treatment}$ (1, 13) = 4.832, P = 0.041]. β-Arrestin-2 deletion resulted in a marked increase of the basal pAkt 308 level in the dorsal striatum (*t*-test, P = 0.027), but chronic alcohol consumption failed to bring any further increase in pAkt 308 or pGSK3β level in the dorsal striatum of β-arrestin-2 KO mice (Fig. 6A, B) [$F_{treatment \times genotype}$

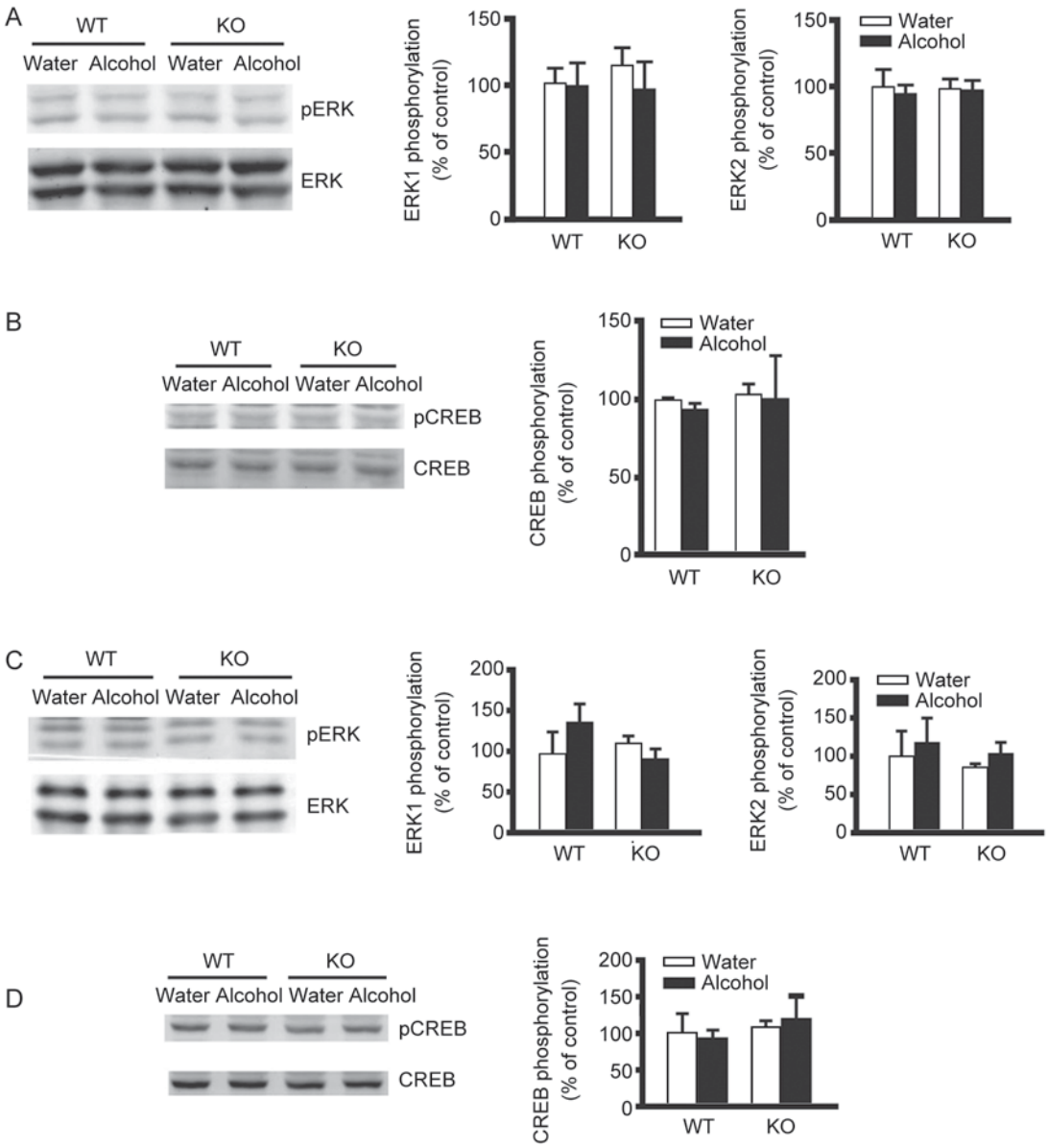

Fig. 7. Long-term alcohol consumption did not change pERK and pCREB levels in the striatum of WT and β-arrestin-2 knockout (KO) mice. After one month of voluntary consumption of 9% alcohol or tap water, the levels of pERK (A, C) and pCREB (B, D) in the dorsal (A, B) and ventral (C, D) striatum from WT and KO mice were determined by Western blot. *n* = 3–4/group. The levels of pERK and pCREB were normalized to total ERK and total CREB respectively, and expressed as a percentage of the WT/Water group. All values are presented as mean ± SEM, two-way ANOVA, *post hoc* Tukey's test.

(1, 24) = 7.060, P = 0.014 for pAkt 308; $F_{\text{treatment} \times \text{genotype}}$ (1, 24) = 1.594, P = 0.224 for pGSK3β]. No changes in Akt phosphorylation at 473 [$F_{\text{treatment} \times \text{genotype}}$ (1, 8) = 0.242, P = 0.636] or total Akt were found in the dorsal striatum of WT and KO mice that drank alcohol compared with those that drank water. The levels of pAkt and pGSK3β were also tested in the ventral striatum of WT and KO mice treated with alcohol, but no difference was detected (Fig. 6C, D). Moreover, no changes in the pERK and pCREB levels were detected in either the dorsal or ventral striatum of both genotypes after one month of alcohol consumption (Fig. 7) [$F_{\text{treatment} \times \text{genotype}}$ (1, 26) = 0.109, P = 0.744 for pERK2 in dorsal striatum; $F_{\text{treatment} \times \text{genotype}}$ (1, 26) = 0.170, P = 0.684 for pCREB in dorsal striatum]. These data indicate that β-arrestin-2 plays an essential role in the regulation of alcohol-induced Akt and GSK3β phosphorylation in the dorsal striatum.

DISCUSSION

The present study provides *in vivo* evidence for the involvement of β-arrestin-2 in alcohol preference and reward by showing that its deletion enhanced alcohol consumption and alcohol-induced CPP scores in mice. β-Arrestin-2 deficiency led to decreased responses to acute alcohol treatment. Also, the activity of Akt and GSK3β increased in the dorsal striatum of WT, but not KO mice during chronic free consumption of alcohol.

Björk *et al*. reported that β-arrestin-2 KO mice display reduced voluntary consumption of a solution containing 6% alcohol and 0.2% saccharin by two-bottle choice with gradually increasing alcohol concentrations from 2% to 6%[24]. In our study, the consumption and preference for alcohol in the ascending concentrations (3, 5, 7, 9, 11, 13 and 15%) were investigated in the two-bottle choice paradigm. The voluntary alcohol consumption of KO mice was significantly greater than that of WT mice, especially at 15%, when there was a robust jump of alcohol intake in the KO mice. No difference in consumption or preference was observed between these two genotypes at ≤7% alcohol. The discrepancy between our study and that by Björk *et al.* may be due to the different designs of the two-bottle choice test. Another factor may be the background of the KO mice used. The mice in our study were back-crossed to C57BL/6J for 10 generations. Moreover, in the study by Björk *et al*., differences between WT and KO mice were found at 6% alcohol, whereas in the present study differences were found at higher doses (from 9% to 15%), which are more likely to be pharmacologically important. Besides, we showed that β-arrestin-2 deficiency increased the CPP scores, which is consistent with our data on voluntary alcohol consumption in the two-bottle choice test. Furthermore, as an important mediator of the GPCR signal pathway, β-arrestin-2 negatively regulates morphine analgesia and promotes morphine tolerance in mice[17-20], so the increased intake of alcohol in KO mice seems unlikely to be due to an elevated tolerance to alcohol. However, a recent human study did not find an association between genetic polymorphisms of β-arrestin-2 and alcohol dependence in a Caucasian population[31]. The association between β-arrestin-2 and alcoholism in other cohorts still needs to be confirmed.

The increased alcohol preference and the decreased alcohol-induced locomotion in β-arrestin-2 KO mice complement previous reports that β-arrestin-2 deficiency increases morphine-induced CPP[20] and decreases acute morphine-stimulated locomotor activity[23]. This profile is also consistent with abundant animal data showing an inverse relation between motivational consumption and sensitivity to alcohol, as well as human data of an inverse relation between alcoholism risk and alcohol sensitivity[32,33]. In addition, in this study the lack of alcohol-evoked hyperlocomotion in KO mice could not be explained by a general impairment in spontaneous locomotor activity as demonstrated by Tukey's HSD *post hoc* analysis: no significant differences between WT and KO mice were found in the basal level of locomotor activity on days 2 and 3 of the test. The previous study from Lefkowitz's group showed hypolocomotion in β-arrestin-2 KO mice. In this study, we also found that β-arrestin-2 KO mice had a decreasing tendency (but without statistical significance) in locomotion on day 1 when they were introduced to a novel open field. This may be due to the difference in the environment where the locomotion was tested and the number of animals used for each test. Our study showed that the change in alcohol clearance rate occurred 60 to 90 min, but not 15 or 30 min, after the acute alcohol injection in KO mice. So the hypolocomotion in KO mice detected 15 min after acute alcohol treatment could not be the consequence of a higher blood-alcohol

clearance rate. These data support the idea that β-arrestin-2 regulates alcohol-related behavioral plasticity.

Previous studies showed that alcohol addiction is a psychiatric disorder associated with dysregulation of signaling systems and protein expression at the cellular level[1]. In this study, elevated pAkt basal activity was detected in the dorsal striatum of β-arrestin-2 KO mice, consistent with other findings that these mice exhibit disruption of the interaction of between Akt and PP2A, dysregulation of Akt/GSK3β signaling in the striatum, as well as abnormality in dopamine-dependent behaviors and the pharmacological effect of lithium[22,34]. The expression level of dopamine D2 receptors in WT and β-arrestin-2 KO mice after chronic administration of alcohol was analyzed in this study by real-time quantitative PCR (2 g/kg i.p., b.i.d. for 14 days). Given that no difference in the mRNA levels of D2 receptors between KO and WT in the saline control groups, it was intriguing that the mRNA level of D2 receptors [$F_{genotype \times treatment}$ (1, 23) = 14.719, P = 0.001] increased two-fold in the dorsal striatum of WT mice after chronic alcohol treatment, but no significant increase was detected in KO mice under the same conditions (data not shown). These data suggest that β-arrestin-2 is involved in alcohol addiction by regulating D2 receptor signaling. Furthermore, our study showed that chronic alcohol consumption significantly increased the activity of Akt and GSK3β in the dorsal striatum of WT mice, but not in β-arrestin-2 KO mice. In addition, the phosphorylation of Akt and GSK3β did not change in the ventral striatum of WT mice after chronic voluntary alcohol consumption. A recent study showed that Akt is activated in the nucleus accumbens in response to acute alcohol injection or withdrawal from excessive alcohol drinking[35]. Another study showed that acute alcohol injection stimulates Akt activation in the striatum, which is suppressed by opioid receptor antagonists but not D2 antagonists[36]. These results indicate that Akt is activated in specific brain areas at different stages of alcohol addiction.

In conclusion, the findings of this study support the hypothesis that β-arrestin-2 functions as a protective molecule to inhibit alcohol preference, likely through regulating the activation of signaling pathways including Akt/GSK3β in the dorsal striatum. β-Arrestin-2 may thus serve as a potential target of therapies for the treatment of alcoholism.

ACKNOWLEDGEMENTS

This work was supported by the National Basic Research Development Program of Ministry of Science and Technology, China (2009CB522006) and the National Natural Science Foundation of China (30901798, 91232307 and 31121061).

Received date: 2012-08-30; Accepted date: 2012-11-15

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