Activation of A_{2B} adenosine receptor protects against demyelination in a mouse model of schizophrenia

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Received January 30, 2022; Accepted March 24, 2022

DOI: 10.3892/etm.2022.11323

Abstract. The purpose of the present study was to explore the effects of A_{2B} adenosine receptor (A_{2B}AR) on learning, memory and demyelination in a dizocilpine maleate (MK-801)-induced mouse model of schizophrenia (SCZ). BAY 60-6583, an agonist of A_{2B}AR, or PSB 603, an antagonist of A_{2B}AR, was used to treat SCZ in this model. The Morris Water Maze (MWM) was utilized to determine changes in cognitive function. Moreover, western blotting, immunohistochemistry and immunofluorescence were conducted to investigate the myelination and oligodendrocyte (OL) alterations at differentiation and maturation stages. The MWM results showed that learning and memory were impaired in SCZ mice, while subsequent treatment with BAY 60-6583 alleviated these impairments. In addition, western blot analysis revealed that myelin basic protein (MBP) and chondroitin sulphate proteoglycan 4 (NG2) expression levels were significantly decreased in MK-801-induced mice, while the expression of G protein-coupled receptor 17 (GPR17) was increased. Additionally, the number of anti-adenomatous polyposis coli clone CC-1/OL transcription factor 2 (CC-1$^+$/Olig2$^+$) cells was also decreased. Notably, BAY 60-6583 administration could reverse these changes, resulting in a significant increase in MBP and NG2 protein expression, and in the number of CC-1$^+$/Olig2$^+$ cells, while GPR17 protein expression levels were decreased. The present study indicated that the selective activation of A_{2B}AR using BAY 60-6583 could improve the impaired learning and memory of SCZ mice, as well as protect the myelin sheath from degeneration by regulating the survival and maturation of OLs.

Introduction

Schizophrenia (SCZ) is a complex neurodevelopmental condition influenced by a range of environmental and genetic variables. According to global burden of disease studies, the global age-standardized point prevalence of SCZ is estimated to be ~0.28% (95% CI, 0.24-0.31) (1). Positive symptoms (such as delusions, hallucinations and behavioral abnormalities) and negative symptoms (such as depression, anxiety and aphasia), as well as cognitive impairments (such as attention deficit, impaired learning and memory ability), are the clinical manifestations associated with SCZ (2,3). To date, the pathogenesis of SCZ remains poorly understood. Numerous studies have demonstrated that the function of myelin and oligodendrocytes (OLs) is critical in the pathophysiology of SCZ. Therefore, a better understanding of OLs may aid in the development of SCZ treatment strategies (4,5). Postmortem studies demonstrated a strong association between aberrant OL development and function, as well as demyelination and the pathogenesis of SCZ (6,7). Moreover, histological examinations of the brain revealed a decrease in the number of hippocampal OLs and neurons in patients with SCZ, and the interaction between OLs and neurons was abnormal (8,9). Additionally, cellular ultrastructural examinations indicated significant pathological changes in SCZ, including OL edema, vacuolation, paucity of ribosomes and mitochondria, and an accumulation of lipofuscin granules in prefrontal white matter (10). Given the apparent role of OLs and related molecules in the pathogenesis of SCZ, it is critical to develop an effective strategy for the early prevention and management of SCZ.

Adenosine is widely distributed and can exert its neuromodulatory effects in the central nervous system (CNS) at low

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Abbreviations: SCZ, schizophrenia; OL, oligodendrocyte; CNS, central nervous system; A_{2B}AR, A_{2B} adenosine receptor; NMDA, N-methyl-D-aspartic acid; MK-801, dizocilpine maleate; MWM, Morris water maze; NG2, chondroitin sulphate proteoglycan 4; GPR17, G protein-coupled receptor 17; MBP, myelin basic protein; CC-1, anti-adenomatous polyposis coli clone CC-1; Olig2, OL transcription factor 2; OPC, OL precursor cell

Key words: schizophrenia, A_{2B} adenosine receptor, myelin sheath, oligodendrocyte, oligodendrocyte precursor cell

concentrations (30-300 nM) through its receptors (11). The adenosine receptors (ARs) belong to the G protein-coupled receptor family, which includes four kinds of receptors, A_1, A_{2A}, A_{2B} and A_3. A_{2B}AR was initially cloned from a human brain in 1992 (12). A_{2B}AR was demonstrated to be broadly distributed in all rat tissues (13), but mainly on neurons and glia in the CNS (14). Additionally, A_{2B}AR is known as a low-affinity receptor due to its modest-to-negligible affinity for adenosine and prototypic agonists (15). Nevertheless, it has been demonstrated that A_{2B}AR expression is selectively upregulated under pathological conditions, such as tissue hypoxia and inflammation, as well as when the adenosine concentration in the tissue increases rapidly (16,17), implying that A_{2B}AR may play a critical role under pathological conditions. Previous studies have demonstrated that the A_{2B}AR plays a crucial role in a variety of neurological diseases, including sensorineural hearing, neurogenic bladder spontaneous activity following spinal cord injury and midazolam-induced cognitive dysfunction (18-20). Furthermore, it was previously demonstrated that A_{2B}AR inhibition could facilitate the remyelination process after hypoxic-ischemic injury (21); however, it is unknown whether A_{2B}AR exerts a role in myelin repair in SCZ. Therefore, it is critical to elucidate the role of A_{2B}AR in SCZ pathophysiology.

Animal models of SCZ are valuable for elucidating the etiology of the disease and for establishing novel therapeutic options. Based on the limited understanding of SCZ, there are mainly three types of animal models available: Developmental models, drug-induced models and genetic models (22). The N-methyl-D-aspartic acid (NMDA) receptor is an ionotropic glutamate receptor (23) whose expression in cerebral cortex is usually lower (24). It has been shown that repeated injection of a high dose of the noncompetitive NMDA receptor antagonist, dizocilpine maleate (MK-801), could be used to induce SCZ in animal models (25). In the present study, behavioral, morphological and biochemical experiments were performed to explore the effect of A_{2B}AR on learning and memory abilities, as well as the myelin sheath.

Materials and methods

Animals and ethics statement. According to the experimental requirements, 40 specific pathogen-free ICR male mice (6-weeks-old; weight, 18-22 g) were obtained from the Experimental Animal Center of Ningxia Medical University (Yinchuan, China). Experimental procedures were conducted following the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 8023, revised 1978) and were approved by the Ethics Committee of Ningxia Medical University (approval no. 2018-006). All the animals were housed at room temperature (22±1˚C), with 40-60% humidity, using a 12 h light-dark cycle and allowed free access to water and food with constant air renewal.

Preparation and grouping of the MK-801-induced SCZ mouse model. A total of 40 mice were randomly divided into four groups (10 mice per group) as follows: Control group (Control), MK-801 group (MK-801), MK-801 plus A_{2B}AR agonist group (MK-801 + BAY) and MK-801 plus A_{2B} AR antagonist group (MK-801 + PSB). The SCZ model was established according to a previously described method (26). Briefly, mice were injected intraperitoneally with MK-801 (0.6 mg/kg/day; cat. no. M107; MilliporeSigma) once a day for 14 consecutive days, and the intervention was performed between 3:00 and 4:00 p.m. every day. The control group was treated with the same volume of normal saline. The optimal dose of A_{2B}AR selective agonist BAY 60-6583 (21,27,28) and A_{2B} AR selective antagonist PSB 603 (21,29) was determined according to previous studies. Intraperitoneal injection of BAY 60-6583 (80 μg/kg/day; cat. no. 910487-58-0; Tocris Bioscience) and PSB 603 (25 μg/kg/day; cat. no. 1092351-10-40; Tocris Bioscience) was administered to the MK-801 + BAY and MK-801+ PSB groups, respectively, every 2 days between day 8 and day 14 of the MK-801 treatment period. The mice in the control and MK-801 groups were intraperitoneally injected with an equal volume of normal saline.

Morris water maze (MWM) experiment. The MWM was mainly used for testing the spatial learning and memory of the rodents. Briefly, a circular swimming pool (80 cm in diameter and 40 cm in depth) was used, and the position of the round escape platform (6 cm in diameter) was fixed at the center of one quadrant of the pool. The water, which was kept at 22±2˚C, was dyed black with ink and mixed evenly to ensure that the platform, which was 1 cm below the surface of the water, was hidden from the mice. Each mouse underwent swimming training for 4 days, four times daily with at least 15 min intervals between, and the directional cruise and spatial probe tests were performed as follows. Mice were gently placed into the water facing the wall at one of the artificially designed four quadrants, and four quadrant tests were completed daily, a timer of 1 min was then started. If the mice found the platform they were kept there for 10 sec and the experiment would end. If the mice failed to find the platform in 1 min, they were guided to find the platform and were allowed to stay on the platform for 15 sec. In the directional cruise test, the mice were allowed to enter the water once in each of the four quadrants to find the platform and were allowed to stay there for 10 sec. In the spatial probe test, the underwater platform was removed, and the number of times the mice crossed the location of the original platform over a period of 1 min was recorded. The small animal behavior records analysis system (Smart 3.0 Premium; Panlab) was simultaneously used to record the trial. Statistical analysis was performed on the obtained data.

Mouse brain tissue preparation. After animal behavior testing and analysis, the mice were decapitated. The cerebral cortex was rapidly dissected and half of the samples were instantly frozen and kept at -80˚C for western blotting. The remaining tissues were fixed in 4% paraformaldehyde for 24 h at 4˚C. Paraffin-embedded tissue sections were used for immunohistochemistry and immunofluorescence staining.

Western blot analysis. Freshly taken brain tissues were weighed and cortical proteins were extracted using lysis buffer (Nanjing KeyGen Biotech Co., Ltd.). Total protein was quantified using a bicinchoninic acid protein assay (Nanjing KeyGen Biotech Co., Ltd.). Western blot analyses were performed as previously described (30). The membranes were incubated with primary antibodies against chondroitin sulphate proteoglycan

4 (NG2; 1:1,000; cat. no. ab12905; Abcam), G protein-coupled receptor 17 (GPR17; 1:1,000; cat. no. 10136; Cayman Chemical Company), myelin basic protein (MBP; 1:1,000; cat. no. ab7349; Abcam), β-tubulin (1:2,000; cat. no. ab009-100; Multi Sciences Biotech) and β-actin (1:5,000; cat. no. TA-09; Origene Technologies, Inc.) overnight at 4˚C. β-tubulin or β-actin was used as an internal loading control. After overnight incubation at 4˚C, HRP-labeled secondary antibodies, goat anti-rabbit IgG (1:5,000; cat. no. A21020), goat anti-rat IgG (1:5,000; cat. no. A21040) and goat anti-mouse IgG (1:5,000; cat. no. A21010) (all Abbkine Scientific Co., Ltd.), were applied to the membranes for a 1-h incubation period at room temperature. Subsequently, the membranes were exposed to a luminescent solution (Omni-ECL Pico Light Chemiluminescence kit; cat. no. SQ202L; Epizyme, Inc.), and the protein bands were quantified using Image-J software (National Institutes of Health; version 1.53). All assays were performed independently and in triplicate.

Immunohistochemical staining. Paraffin brain sections 5 μm in thickness were heated for 1 h at 60˚C and then dewaxed with xylene at room temperature. The sections were subsequently rehydrated with a descending alcohol series at room temperature (anhydrous ethanol I, 5 min; anhydrous ethanol II, 5 min; 95% alcohol, 5 min; 85% alcohol, 5 min; and 75% alcohol, 5 min). The sections were hydrated with PBS for 3 min. Next, the sections were placed in a microwave for antigen retrieval. After incubation in 0.3% hydrogen peroxide to deactivate endogenous peroxidase for 20 min at 37˚C, sections were blocked with 1% goat serum (MilliporeSigma) and 0.3% Triton X-100 (MilliporeSigma) for 30 min at 37˚C. Subsequently, the brain sections were incubated with primary antibodies against MBP (1:200; cat. no. ab7349; Abcam) and NG2 (1:200; cat. no. ab129051; Abcam) overnight at 4˚C. The next day, sections were incubated with HRP-conjugated goat anti-rabbit IgG (1:400; cat. no. A21020; Abbkine Scientific Co., Ltd.) and goat anti-rat IgG (1:400; cat. no. A21040; Abbkine Scientific Co., Ltd.) secondary antibodies for 1 h at room temperature. Following three washes using PBS, the sections were counterstained with DAB for 5 min and hematoxylin for 2 min at room temperature for observation. The cerebral cortex was identified and images were captured after observation by brightfield microscopy. The observers were blinded to the experimental groups. All assays were performed independently and in triplicate.

Immunofluorescence staining. The brain slices were heated for 1 h at 60˚C and then dewaxed with xylene at room temperature. The sections were subsequently rehydrated with a descending alcohol series at room temperature (anhydrous ethanol I, 5 min; anhydrous ethanol II, 5 min; 95% alcohol, 5 min; 85% alcohol, 5 min; and 75% alcohol, 5 min). The sections were hydrated with PBS for 3 min. Following antigen retrieval using a microwave for 10 min, sections were blocked with 1% goat serum in 0.3% Triton X-100 for 30 min at 37˚C. Subsequently, the brain sections were incubated with primary antibodies against the anti-adenomatous polyposis coli clone CC-1 (CC-1; 1:100; cat. no. OP80-100UG; Merck KGaA) and OL transcription factor 2 (Olig2; 1:100; cat. no. ab109186; Abcam) overnight at 4˚C. Following three washes using PBS, the sections were incubated with a fluorescent secondary antibody mixture of goat anti-rabbit IgG (Dylight 594; 1:300; cat. no. A23420; Abbkine Scientific Co., Ltd.) and goat anti-mouse IgG (Dylight 488; 1:300; cat. no. A23210; Abbkine Scientific Co., Ltd.) for 1 h at room temperature. Finally, all brain sections were counterstained with DAPI for 5 min at room temperature. Images of the cerebral cortex were captured using a fluorescence microscope. An observer blind to the present study counted the number of co-labeled positive cells by using ImageJ software (National Institutes of Health, version 1.53). All assays were performed independently and in triplicate.

Statistical methods. SPSS (version 22.0; IBM Corp.) and GraphPad software (version 8.0; GraphPad Software, Inc.) were used to perform all statistical analyses. The data are presented as the mean ± standard error. The differences between groups were analyzed using one-way ANOVA followed by Tukey's post hoc test. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Protective effect of BAY 60-6583 on animal learning and memory ability in an MK-801-induced SCZ mouse model. MK-801 (0.6 mg/kg/day) was intraperitoneally injected for 14 consecutive days to establish the SCZ model, BAY 60-6583 or PSB 603 was administered every 2 days between day 8 and day 14 of the MK-801 treatment period (Fig. 1A). The results showed that the body weight of the MK-801 group significantly decreased ($P<0.05$), with BAY 60-6583 and PSB 603 treatment failing to rescue the slow body weight gain ($P>0.05$) (Fig. 1B). Next, the MWM test was utilized, and as shown by the trajectory chart (Fig. 1C), the escape latency of the SCZ mice in the directional cruise test was notably prolonged ($P<0.05$) (Fig. 1D). In comparison, BAY 60-6583 treatment significantly reduced the escape latency of the mouse model ($P<0.05$) (Fig. 1D). Notably, in the spatial probe test, the number of crossings of the MK-801 group was markedly lower compared with that of the control group ($P<0.05$) (Fig. 1E), while subsequent BAY 60-6583 treatment notably increased the number of crossings compared with the MK-801 group ($P<0.05$) (Fig. 1E). Furthermore, there were no significant differences in terms of crossings and escape latency between the MK-801 + PSB and the MK-801 groups ($P>0.05$) (Fig. 1D and E).

Effect of BAY 60-6583 on the maturation of OLs in the SCZ mouse model. Immunohistochemical analysis demonstrated that the area stained for MBP, a specific marker for myelin sheath (31,32), in the cerebral cortex (Fig. 2A) of the MK-801 group was lower than that in the control group ($P<0.05$) (Fig. 2B and C). Moreover, BAY 60-6583 administration significantly alleviated the decrease in MBP in the cerebral cortex of the mouse model, displaying higher neurite density and arborization in the cortex, with better shape and a dense distribution (Fig. 2B and C), while no changes in MBP staining were found in the MK-801 + PSB group compared with that in the MK-801 group ($P>0.05$) (Fig. 2B and C). Consistent with the immunohistochemistry results, western blotting

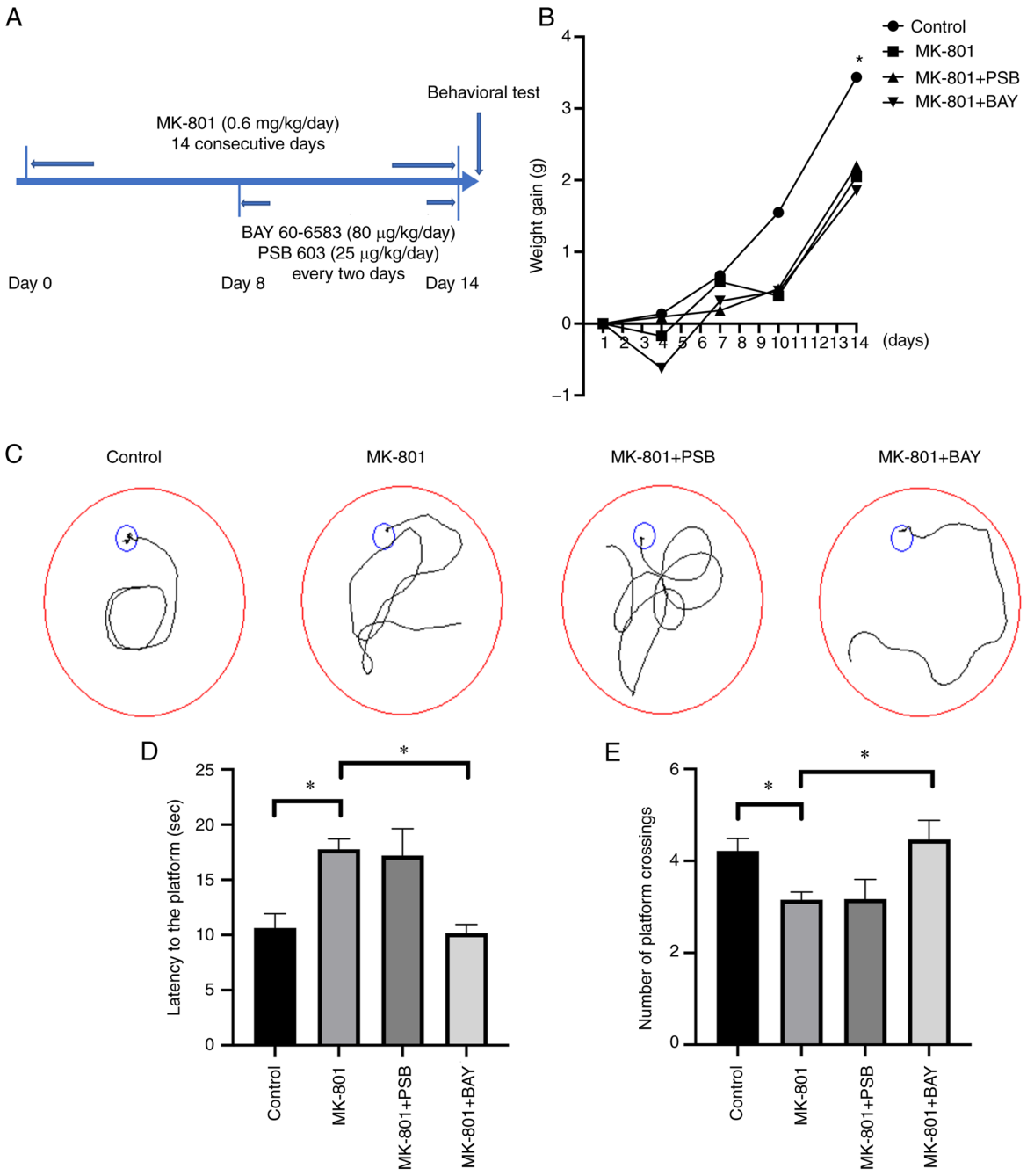

Figure 1. Impaired learning and memory ability is restored following BAY 60-6583 treatment in SCZ mice. (A) MK-801 was administered via intraperitoneal injection for 14 consecutive days to establish the SCZ mouse model. On the 8th day, BAY 60-6583 (80 μg/kg/day) or PSB 603 (25 μg/kg/day) was administered via intraperitoneal injection every 2 days. (B) Changes in body weight during MK-801 treatment. The MK-801 group, the MK-801 + PSB group, the MK-801 + BAY group were compared with the control group as indicated by the asterisk. (C) A representative trace diagram of mice in the MWM test. (D) The mice in the MK-801 group had a notably longer escape latency (time taken to locate the submerged platform) than the control mice, and this was reversed by BAY 60-6583 administration. (E) The number of platform crossings in the MWM test were reduced in the MK-801 group. BAY 60-6583 treatment significantly increased the number of platform crossings. Data are presented as the mean ± SEM. *P<0.05. SCZ, schizophrenia; MK-801, dizocilpine maleate; A_{2B}AR, A_{2B} adenosine receptor; BAY 60-6583, A_{2B}AR agonist; PSB 603, A_{2B}AR antagonist; MWM, Morris water maze.

revealed that the expression levels of MBP in the brain of the MK-801 group were markedly decreased compared with those in the control group (P<0.05) (Fig. 2D and E). By contrast, a significant increase in MBP expression levels was observed in the MK-801 + BAY group compared with the model group (P<0.05) (Fig. 2D and E), whereas there was no difference in MBP expression levels in the MK-801 + PSB group compared with those in the MK-801 group (P>0.05) (Fig. 2D and E).

BAY 60-6583 protects the survival of OL precursor cells (OPCs) and the differentiation of OLs in the SCZ mouse model. Immunohistochemical analysis further revealed that the number of OPCs that were positive for NG2 in the cerebral cortex was markedly decreased in the MK-801 group compared with that in the control group (Fig. 3A and B) (P<0.05). Subsequent BAY 60-6583 administration notably increased the number of $NG2^+$ cells in the cerebral cortex compared with that in the MK-801 group (P<0.05) (Fig. 3A and B).

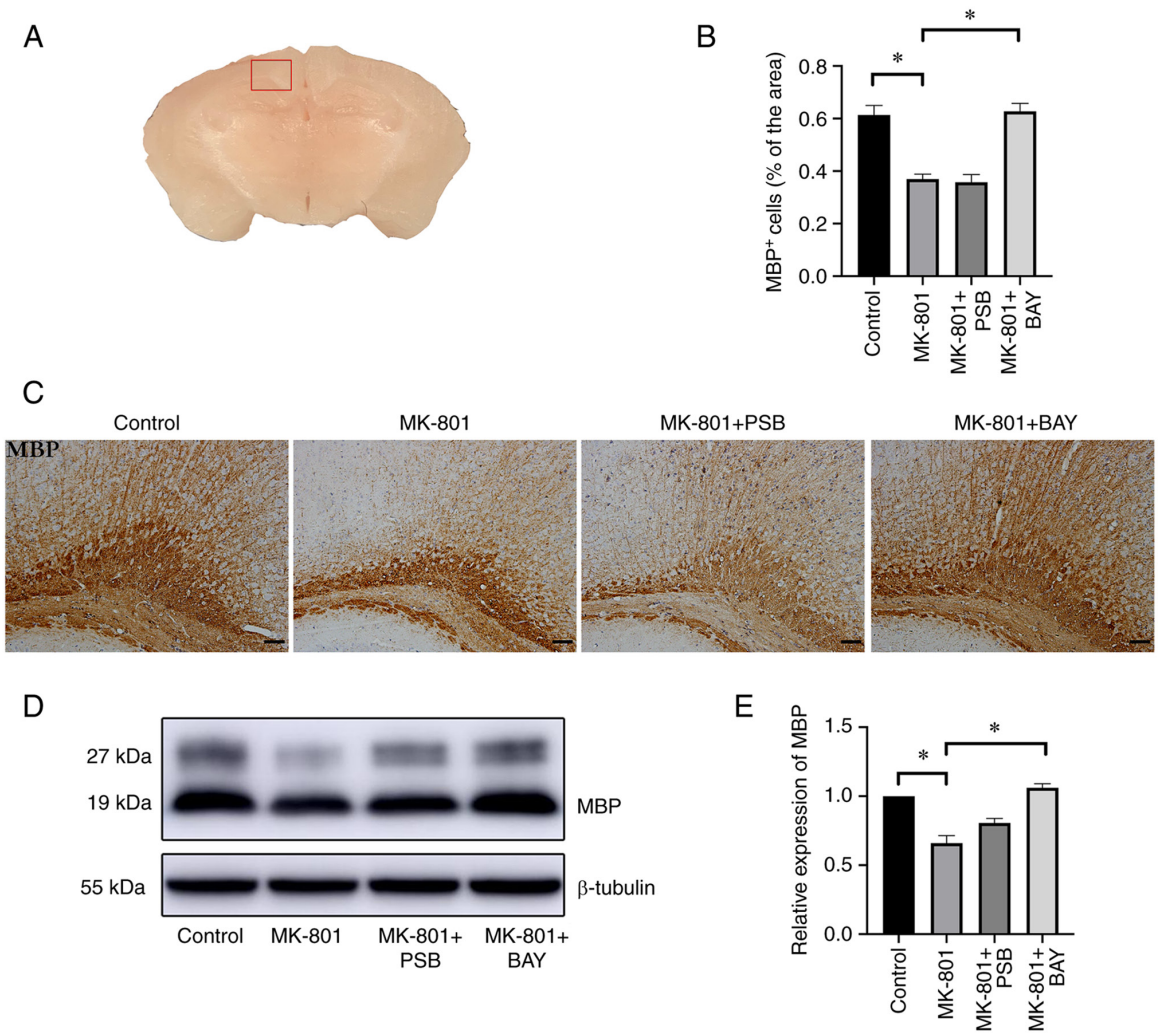

Figure 2. Myelination process is modified by A_{2B}AR activation in SCZ mice. (A) The square shows the area under investigation is the posterior cingulate cortex of the mouse brain. (B) MK-801 markedly reduced the percentage of MBP$^+$ staining in the cerebral cortex, while BAY 60-6583-treated SCZ mice showed a notable increase in MBP$^+$ staining. (C) A representative image of MBP immunohistochemical staining in the cerebral cortex (scale bar=50 μm). (D) MBP protein expression levels were measured using western blotting. (E) MBP protein expression was downregulated in the MK-801 group compared with that in the control group, while increased expression was observed in the MK-801+BAY group compared with that in the MK-801 group. Data are presented as the mean ± SEM. *P<0.05. A_{2B}AR, A_{2B} adenosine receptor; SCZ, schizophrenia; MBP, myelin basic protein; MK-801, dizocilpine maleate; BAY 60-6583, A_{2B}AR agonist; PSB 603; A_{2B}AR antagonist.

Consistently, western blot analysis showed that the protein expression levels of NG2 were reduced, while those of GPR17 were increased in the MK-801 group compared with those in the control group (P<0.05) (Fig. 3C-F). Activation of A_{2B}AR (BAY 60-6583 treatment) resulted in an increase in the protein expression levels of NG2 in the MK-801+BAY group (P<0.05) (Fig. 3C and D), while the protein expression levels of GPR17 were markedly decreased (P<0.05) (Fig. 3E and F). CC-1 is a specific marker of mature OLs that is often co-labeled with Olig2, representing mature OLs (33). Immunofluorescent double staining for CC-1/Olig2 revealed low levels in the cerebral cortex of the MK-801 group, and a lower number of mature OLs compared with that in the control group (P<0.05) (Fig. 3G and H). Meanwhile, immunofluorescence revealed that A_{2B}AR activation resulted in increased CC-1/Olig2 levels in the cerebral cortex region of the MK-801 + BAY group (P<0.05) (Fig. 3G and H). Similarly, there was no change in the number of cells positive for CC-1/Olig2 in the cerebral cortex of the MK-801 + PSB group (P>0.05) (Fig. 3G and H).

Discussion

The present study aimed to investigate the regulatory effects of A_{2B}AR on the cognitive behavior and myelin sheath degeneration in mice, for which a SCZ mouse model was established. The glutamatergic system in SCZ is usually abnormal, characterized by the glutamatergic signal transduction dysfunction of the NMDA receptor (34-36). The delivery of NMDA receptor antagonist not only elicits an SCZ-like behavioral change in healthy animals, but also causes further SCZ-like brain pathological changes, such as OL injury, demyelination, and GABAergic and dopaminergic system disorders (37). This is why SCZ animal models treated with NMDA receptor antagonists have been widely used to investigate SCZ pathogenesis. The present study established an SCZ mouse model by intraperitoneal injection of MK-801, an NMDA receptor antagonist, for 14 consecutive days. As a result, the mice exhibited hyperlocomotion, ataxia, hindlimb abduction, flat body posture, stereotyped head-rotation behavior, spontaneous

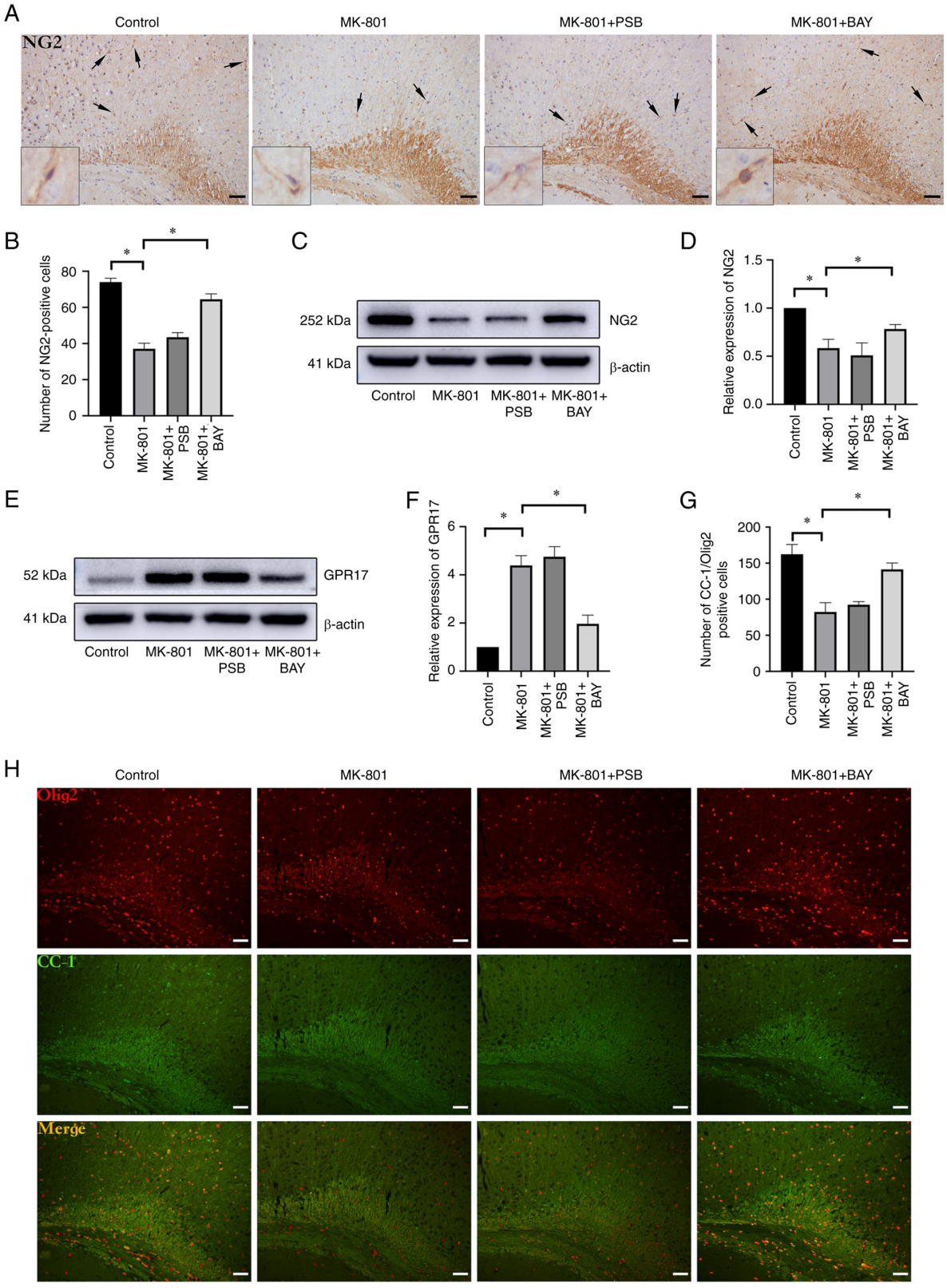

Figure 3. Survival and maturation of OLs are modified by A_{2B}AR activation in SCZ mice. (A) Immunohistochemical images of $NG2^+$ OPC staining in the cerebral cortex (scale bar, 50 μm) The arrows and insert box indicate the $NG2^+$ cells; the inset boxes are at x8 magnification. (B) MK-801 treatment markedly reduced the number of $NG2^+$ OPCs in the cerebral cortex. Administration of BAY 60-6583 in the SCZ mice notably increased the number of $NG2^+$ OPCs in the cerebral cortex. (C) Representative immunoblots of the NG2 protein levels. (D) MK-801-treated mice showed a significant reduction in NG2, while BAY 60-6583 administration increased NG2 protein levels in the cerebral cortex. (E) Representative immunoblots of GPR17 protein expression levels. (F) GPR17 protein expression levels were significantly increased in MK-801-treated mice and were restored in MK-801 + BAY-treated mice. (G) The number of $CC\text{-}1^+/Olig2^+$ cells was reduced in the MK-801 group, while a significant increase was observed in the MK-801 + BAY group. (H) Immunofluorescence staining was performed for CC-1/Olig2 in the cerebral cortex (scale bar, 50 μm). Data are presented as the mean ± SEM. $^*P<0.05$. OLs, oligodendrocytes; A_{2B}AR, A_{2B} adenosine receptor; SCZ, schizophrenia; NG2, chondroitin sulfate glycoprotein 4; OPCs, OL precursor cells; MK-801, dizocilpine maleate; GPR17, G protein-coupled receptor 17; CC-1, anti-adenomatous polyposis coli clone CC-1; Olig2, OL transcription factor 2; BAY 60-6583, A_{2B}AR agonist; PSB 603; A_{2B}AR antagonist.

locomotor activity and gradual weight gain. These findings were consistent with other previous findings (38,39). In addition, a significant decrease in learning and memory abilities was observed in the SCZ mice. Notably, selective activation of A_{2B}AR could alleviate the learning and spatial memory impairment in the SCZ mice.

Previous studies have demonstrated the following: i) Demyelination is closely associated with the cognitive and learning dysfunction in SCZ (40-42); ii) OL and myelin dysfunction are considered primary changes in SCZ, not purely as secondary consequences of the illness or treatment (43); and iii) enhancing remyelination could alleviate SCZ-like symptoms (30). Based on this theory, the changes in MBP levels in the cerebral cortex of SCZ mice were initially investigated in the present study. Unexpectedly, a marked decrease in MBP expression levels was detected using both western blot and immunostaining analysis. This finding implies that the development of SCZ is most likely related to aberrant myelination. Xiu *et al* (44) also showed that MK-801 could cause loss of myelin fibers in the cerebral cortex of SCZ mice. Likewise, in the present study, the administration of BAY 60-6583 significantly elevated MBP expression levels in SCZ mice, suggesting that BAY 60-6583 could facilitate myelin repair, leading to the improvement of cognitive function in SCZ.

Myelin regulation is a dynamic process in which both freshly generated and pre-existing OLs remyelinate to allow learning and plasticity, or respond to activity (45). In the CNS, OLs are derived from mitotically active and migratory OPCs, which undergo stepwise genotypic and phenotypic differentiation processes before myelin formation. Accordingly, we hypothesized that the abnormal myelination was mainly due to myelin loss or abnormal development of OPCs. As expected, the delivery of MK-801 decreased the number of $NG2^+$ cells and decreased NG2 protein expression levels in the cerebral cortex. Although previous studies have shown an increase in $NG2^+$ cells migrating to the lesion site after myelin damage (46), MK-801 has also been shown to have a general cytotoxic impact on brain tissues (47,48), with OLs appearing to be more affected by MK-801 treatment than other cell types (49). Myelin injury in the brains of MK-801-induced SCZ mice is considered to be caused by aberrant OPCs. Additional experiments in the present study revealed that BAY 60-6583 treatment could markedly increase the number of $NG2^+$ cells and boost NG2 protein expression in the SCZ model mice. Due to the limited number of OPCs in the brain, increased OPC numbers are necessary for myelin repair. Meanwhile, the demyelination or myelin destruction caused by various factors may be incurable without replenishing the OLs derived from OPCs. To that end, the differentiation of OPCs after treatment with BAY 60-6583 or PSB 603 was further investigated and the number of $CC\text{-}1^+/Olig2^+$ cells was found to be markedly reduced in SCZ mice. BAY 60-6583 could significantly increase the number of $CC\text{-}1^+/Olig2^+$ cells in the SCZ mice, implying that BAY 60-6583 may promote the differentiation and maturation of OLs. These findings were supported by the change in GPR17 expression levels between the two groups. That is, BAY 60-6583 significantly reduced the increase in GPR17 expression caused by MK-801. GPR17 acts as a suppressor of OL differentiation and myelination (50). GPR17 knockout in the CNS can facilitate OPC differentiation and maturation (51,52). GPR17 inhibition can enhance the maturation of primary rat and mouse OLs, efficiently favor human OL differentiation (53) and accelerate the remyelination process after lysolecithin-induced demyelinating injury (54). This suggests that the impaired differentiation and maturation of OLs is closely associated with MK-801-mediated brain demyelination in SCZ mice and that the A_{2B}AR agonist, BAY 60-6583, likely promotes the differentiation and maturation of OLs.

Moreover, the present study showed that cognitive behavior and brain demyelination did not deteriorate in SCZ mice treated with PSB 603. Theoretically, A_{2B}AR is an AR with a low affinity for adenosine that is activated by a high quantity of adenosine. The concentration of adenosine produced by the brains of SCZ mice is insufficient to activate A_{2B}AR.

In conclusion, the present study established A_{2B}AR as a possible therapeutic target for reversing demyelination in a SCZ mouse model. The data from this study corroborated prior findings by demonstrating that A_{2B}AR activation could alleviate SCZ symptoms caused by myelin degeneration and reverse learning and memory impairments in SCZ mice. Notably, the present study revealed that GPR17 might be involved in A_{2B}AR-mediated differentiation and maturation of OLs and protection of the myelin sheath, thus providing new targets for the treatment of SCZ. However, the present study is limited by the absence of further behavioral tests for SCZ-like symptoms, and additional mechanisms require investigation. Therefore, further in-depth studies are warranted in the future.

Acknowledgements

Not applicable.

Funding

This study was supported by the CAS 'Light of West China' Program (grant no. XAB2017AW05), the National Natural Science Foundation of China (grant nos. 31360240 and 82060238), the Ningxia Natural Science Foundation (grant nos. NZ17072 and 2022AAC03155) and the 'Chunhui Plan' of the Ministry of Education (grant no. Z2016063).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

QM and DW confirm the authenticity of all the raw data. DW, YiL, JW and JLi performed the experiments. QM, JLiu and JS contributed to the conception of the study and supervised the graduate students. DW, YuL and HY contributed to the acquisition and interpretation of data. HY and JS contributed to the revision of the manuscript. QM, JS and HY contributed to obtaining funding, designed the project and gave final approval of the version to be published. DW and YuL wrote the manuscript and analyzed the data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All experimental procedures and protocols used in this study were reviewed and approved by the Ethics Committee for the Use of Experimental animals at Ningxia Medical University (Yinchuan, China; approval no. 2018-006).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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