

TREK-1 Mediates The Alleviative Effects of GABAB Receptor Antagonism on Depression-Like Behavior in Chronic Unpredictable Stress Rats

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Abstract

GABA_B receptor (GABA_B R) antagonists are known to have antidepressant effects. TWIK-related potassium channel-1 (TREK-1) plays a role in GABA_B R signaling. However, the role of TREK-1 in the antidepressant actions of GABA_B R antagonist is still unclear. This study aimed to investigate whether TREK-1 mediates the antidepressant actions of GABA_B R antagonist. To investigate this hypothesis, chronic unpredictable stress rats were treated with a GABA_B R antagonist, GABA_B R agonist, or TREK-1 blocker. Depression-like behavior was assessed by open field tests and sucrose preference tests. GABA_B R and TREK-1 protein levels were measured by western blotting. The results demonstrated that the GABA_B R antagonist alleviated depression-like behavior and reversed the decrease in hippocampal TREK-1 protein expression that characterizes chronic unpredictable stress rats. Conversely, the GABA_B R agonist exacerbated depression-like behavior and further decreased hippocampal TREK-1 protein expression. In addition, the TREK-1 blocker alleviated the depression-like behavior of chronic unpredictable stress rats and increased hippocampal TREK-1 protein expression. These results suggest that the alleviative effects of the GABA_B receptor antagonist on depression-like behavior in chronic unpredictable stress rats are at least partially mediated through TREK-1. These novel findings will be helpful for the clinical therapy of depression.

Introduction

Depression, a common and severe neuropsychiatric disease, is one of the leading causes of global disease burden (Chong et al. 2019). It is caused by genetic and environmental factors (Llorente et al. 2018). Depression-like behavior is core symptom in depression (Höflich et al. 2019). Moreover, anhedonia can be used as a marker to judge the degree of depression and the effect of drug treatment (Höflich et al. 2019). The chronic unpredictable stress rat model is commonly used for the study of depression. In this model, a series of unpredictable stress stimuli are administered to the rats, which causes them to show depression-like behavior. GABA_B receptor (GABA_B R) dysfunction is involved in the pathogenesis of depression (Jacobson et al. 2018). Blockade of GABA_B Rs can show an antidepressant effect (Pytka et al. 2016). GABA_B Rs are divided into autoreceptors and heteroreceptors based on their function. Autoreceptors regulate the release of GABA through negative feedback (Kobayashi et al. 2012). Heteroreceptors act on non-GABAergic neurons and inhibit the release of noradrenaline, serotonin, dopamine (Gassmann et al. 2012), and glutamate (Evenseth et al. 2020). The downstream signaling pathways of GABA_B Rs include adenylyl cyclase (AC) through the G_{i/o} signaling pathway (Evenseth et al. 2020; Gassmann et al. 2012), protein kinase A (PKA) signaling pathway, voltage-gated Ca²⁺ channels (VGCC) signaling pathway (Gassmann et al. 2012), soluble N-ethyl maleimide-sensitive factor attachment protein receptor (SNARE) signaling pathway (Gassmann et al. 2012), G-protein coupled inwardly rectifying K⁺ channel (GIRK) signaling pathway (Bettler et al. 2004; Misgeld et al. 1995), and extra-cellular signal-regulated protein kinase 1 and 2 (ERK1/2) signaling pathway (Tu et al. 2007). Wagner and Dekin et al. suggested that GABA_B Rs could control multiple types of K⁺ channels (Lüscher et al. 1997; Wagner and

Dekin 1993). Moreover, Sandoz et al. suggested in mice with genetic knockout of Kir3 that the Kir3 potassium channel was not the only potassium channel contributing to GABA_B R inhibition (Sandoz et al., 2012).

TWIK-related potassium channel-1 (TREK-1) is a novel antidepressant target (Gordon and Hen 2006; Heurteaux et al. 2006; Honoré 2007). Blockers of TREK-1 (Ye et al. 2015), such as spadin and analogs of spadin, have a significant antidepressant-like response (Djillani et al. 2019). TREK-1 knockout mice display a depression-resistant phenotype (Heurteaux et al. 2006). TREK-1 is present throughout the brain and spinal cord, and is expressed in the dendrites of neurons (Pineda et al. 2019). TREK-1 produces background leak-type potassium currents that regulate resting membrane potential and levels of cellular excitability (Pineda et al. 2019; Zhang et al. 2018). Deng et al. revealed that GABA_B R activation exerted an inhibitory effect on neuronal excitability by mediating the TREK-2 (Deng et al. 2009). TREK-1 and TREK-2 are two-pore-domain potassium (K₂P) channel proteins, and they share 78% homology in their genomic sequences (Lesage et al. 2000). Furthermore, Sandoz et al. revealed that TREK-1 was an additional target of GABA_B R in the hippocampus by using a photoswitchable conditional subunit (Sandoz et al. 2012). Based on the above studies, we speculated that TREK-1 mediates the alleviative effects of GABA_B receptor antagonism on depression-like behavior in chronic unpredictable stress rats. To investigate this hypothesis, the GABA_B R and TREK-1 proteins were measured by western blotting, and depression-like behavior was assessed by open field tests (OFT) and sucrose preference tests (SPT).

Materials And Methods

2.1 Grouping and drug administration

Three-month-old adult male Sprague-Dawley (SD) rats (250 ± 50 g, n=59) were obtained from Dalian Medical University. This work was permitted by the Animal Experiments Ethical Committee of Dalian Medical University (AEE19087). The rats were placed in rearing cages (50 cm × 30 cm × 20 cm). The temperature of the rearing environment was 21 ± 1°C, the humidity was 40%-70%, and the rearing environment was dry, clean, and ventilated. The rats were exposed to a 12 h/12 h constant light/dark cycle. The rats were provided normal food and water (n=4 rats/cage). All the rats were adapted to the rearing environment for a week. The experimental rats were divided into nine groups (n=6-8 rats/group). The groups included the control group (C), three drug treatment only groups (C+Baclofen, C+CGP55845, and C+Spadin), the CUS group, and three drug intervention groups (CUS+Baclofen, CUS+CGP55845, CUS+Spadin, and CUS+Citalopram). The chronic unpredictable stress treatments were performed according to a previously described modified protocol (Ge et al. 2020; Katz et al. 1981; Yue et al. 2018). The model group was subjected to one kind of stimulation per day for 27 days, and none of the stimuli appeared twice on two consecutive days. The types of stimulation included restraint (30 min), fasting (24 h), water deprivation (24 h), warm water swimming (15 min, 31°C), warm water swimming (15 min, 31°C), cold water swimming (10 min, 18°C), social isolation (24 h), wet mattress + 30° inclined cage (24 h), tail suspension (1 centimeter from the root of the tail, 10 min), and continuous overnight lighting. After

the establishment of the depression model, the open field tests and sugar preference tests were used to test the depression-like behavior. The rats without chronic unpredictable stress treatments were tested at the same time. In addition, The drug administration was performed for 4 weeks, during which the chronic stress stimulation was carried out. The route of administration was intraperitoneal injection. The rats without drug treatments were given to the corresponding volume of physiological saline solution (vehicle). The dosages of the intervention drugs were based on the references and pilot study. The dosage of the GABA_B R agonist (baclofen, Target Mol Company) was 3 mg/kg/d, the dosage of the GABA_B R antagonist (CGP55845, APExBIO Company, USA) was 0.1 mg/kg/d, the dosage of the TREK-1 blocker (spadin, APExBIO Company, USA) was 0.1 mg/kg/d, and the dosage of the positive control drug, a 5-hydroxytryptamine (5-HT) reuptake inhibitor (citalopram, MedChem Express, USA), was 5 mg/kg/d.

2.2 Open field test (OFT)

A SuperMaze V2.0 animal behavior video analysis system (Shanghai Xinruan Technology Co., Ltd.) was used to evaluate the behavior of the rats in the open field test. The autonomous behavior and exploratory behavior of the rats in the novel environment were recorded and analyzed. The rat was placed in the center of the box (50 cm × 50 cm × 40 cm), and video was recorded for 5 min. The inner wall of the box was black, and the bottom surface was divided into a lattice containing 25 squares (10 cm × 10 cm per square) for analysis. The light was approximately 40 lx. The background noise of the laboratory was below 65 dB. The total time spent and the total distance moved at the central region of the open field were analyzed for each rat. To avoid the influence of odor, 75% alcohol was used to clean the cages after each test was performed.

2.3 Sucrose preference test (SPT)

The sucrose preference test is the most commonly used assessment method for the symptoms of depression (Höflich et al. 2019; Tao et al. 2019). A sucrose preference test was performed to evaluate the anhedonia level (Liu et al. 2018; Tao et al. 2019). During the adaptation period, the rats were provided 2 bottles of 1% sucrose solution on the first day. On the second day, the rats were provided 1 bottle of water and 1 bottle of 1% sucrose solution. The positions of the two bottles were switched after 12 hours. The rats were provided 2 bottles of water on the third day. One day after the sucrose adaptation period, a sucrose preference tests were conducted. All rats were provided 1 bottle of water and 1 bottle of 1% sucrose solution. After 12 hours, the positions of the two bottles were switched. The consumption volumes of the rats were measured for 24 hours. The sucrose preference value was evaluated according to the following formula (Wang et al. 2019): $\text{Sucrose preference} = \frac{\text{sucrose consumption}}{[\text{sucrose consumption} + \text{water consumption}]} \times 100\%$.

2.4 Western blotting

Hippocampal samples were isolated according to The Brain Atlas by using a brain mold. The hippocampal protein was extracted according to the instructions of the Ketyl Total Protein Extraction Kit (Nanjing Kaiji Biotechnology Development Co., Ltd). The target protein concentration was evaluated by

using the BCA Protein Content Detection Kit (Cat: KGPBCA, Nanjing Kaiji Biotechnology Development Co., Ltd.). Denatured protein (30 µg/well) was loaded into a 7.5% sodium dodecyl sulfate gel and measured by polyacrylamide gel electrophoresis. Afterwards, the target proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were incubated with 5% skim milk for 2 h at 37°C. After that, the PVDF membranes were immunoblotted with the following antibodies: GABA_B R-Ab (primary antibody, 1:5000, Abcam Co., Ltd.), TREK-1-Ab (primary antibody, 1:1000, BioVision), and GAPDH-Ab (primary antibody, 1:1000, ImmunoWay Company). Tris-buffered saline containing Tween 20 (TBST) was used to wash the membranes (10 min × 3); afterward, the membranes were placed in an incubation box with horseradish peroxidase (HRP)-labeled secondary antibody (anti-goat IgG/HRP, 1:5000, ZSJQ-BIO Company, Beijing, China; anti-mouse, IgG/HR, 1:5000, ZSJQ-BIO Company, Beijing, China) at room temperature for 2 h. Subsequently, the membranes were washed in TBST (10 min × 3), and Bio-Rad analysis software (Hercules, CA, USA) was used to measure the signal intensities of the protein bands. TREK-1 and GABA_B R protein expression was normalized to GAPDH (n=5/group).

2.5 Statistics

In the present study, SPSS 23.0 (Aramonk, NY, USA) was used. All data are presented as the means ± standard deviation. One-way ANOVA was used for comparison of samples among multiple groups. Differences between two groups were analysed using Student's t-tests. To evaluate the possible interaction effect of chronic unpredictable stress and drug treatments on depressive-like behavior and GABA_B R and TREK-1 protein expression among rats, two-way ANOVA was used. $p < 0.05$ was regarded as statistically significant. The double-blind method was used in this study.

Results

3.1 Both the GABA_B R antagonist and the TREK-1 blocker alleviate the depression-like behavior of chronic unpredictable stress (CUS) rats

In the open field test (Fig.1 A), the CUS rats showed decreased exploratory behavior. Compared with the control rats, the CUS rats exhibited decreased time spent in the center area. In addition, the GABA_B R antagonist, TREK-1 blocker, and positive control (citalopram) treatments alleviated the decreased exploratory behavior of the CUS rats. The above results demonstrated that the autonomous behavior and exploratory behavior of the rats were weakened in a new environment under unpredictable chronic stress. The TREK-1 blocker, GABA_B R antagonist, and 5-hydroxytryptamine reuptake inhibitor (positive control) treatments alleviated the above abnormal behaviors, while treatment with the GABA_B R agonist exacerbated the above abnormal behaviors.

In the sucrose preference test (Fig.1 B), compared with that in the control group, the ratio of sucrose solution consumption was significantly decreased in the CUS group, and there were no significant differences between the control group and the drug treatment only groups. Compared with that in the CUS group, the ratio of sucrose solution consumption was significantly increased in the CUS+CGP55845

group, CUS+Spadin group, and CUS+Citalopram group, and the ratio of sucrose solution consumption decreased significantly in the CUS+Baclofen group. These results indicated that the demand for sucrose solution was significantly decreased in the CUS rats. The TREK-1 blocker, GABA_B R antagonist, and positive control treatments increased the demand for sucrose solution in the CUS rats, while treatment with the GABA_B R agonist reduced the consumption of sucrose solution in the CUS rats.

3.2 Both the GABA_B R antagonist and the TREK-1 blocker alleviate the low TREK-1 protein expression in the hippocampus of chronic unpredictable stress (CUS) rats

In the western blotting, hippocampal TREK-1 protein expression decreased significantly in the CUS group compared with the control group. The spadin, CGP55845, and citalopram treatments all alleviated the above decrease in hippocampal TREK-1 protein expression in the CUS rats. However, baclofen exacerbated the decreased TREK-1 protein expression in the CUS rats. In addition, hippocampal GABA_B R protein expression also decreased significantly in the CUS group compared with the control group. Only CGP55845 alleviated the above decrease in hippocampal GABA_B R protein expression in the CUS rats, whereas baclofen increased the hippocampal GABA_B R protein expression in the CUS rats, and spadin and citalopram showed no impact on the above decrease in hippocampal GABA_B R protein expression in the CUS rats. The mean optical density values of GABA_B R and TREK-1 protein expression in the hippocampus of the rats are shown in Fig. 2.

Discussion

4.1 GABA_B R and depression

GABA_B R antagonists are involved in antidepressant-like activity (Evenseth et al. 2019; Fogaça and Duman 2019). GABA_B R knockout mice and mice administered GABA_B R antagonists show an antidepressant-like phenotype (Fogaça and Duman 2019; Luscher et al. 2011). GABA_{B1b}^{-/-} mice and GABA_{B1a}^{-/-} mice show an antidepressant-like phenotype with increased hippocampal neurogenesis (Giachino et al. 2014). GABA_B Rs can contribute to depression through presynaptic or postsynaptic mechanisms (Evenseth et al. 2020). It has been found that the antidepressant effect of CGP56433A can be eliminated via depletion of serotonin by the tryptophan hydroxylase inhibitor chlorophenylalanine (PCPA). It has been suggested that GABA_B R antagonists may play an antidepressant role by interacting with the serotonin system (Slattery et al. 2005). Consistent with the above studies, the results of this study demonstrate that the GABA_B R antagonist alleviated depression-like behaviors in the CUS rats, whereas the GABA_B R agonist exacerbated depression-like behaviors in the CUS rats. The findings of this study and the above published reports provide evidence to support that GABA_B Rs are key regulatory molecular targets for depression-like behavior. The findings of this study and the above published reports provide evidence indicating that GABA_B Rs should be considered the key molecular targets for regulating depression-like behavior.

3.2 TREK-1 and depression

TWIK-related potassium channel-1 (TREK-1) is a potential therapeutic target for depression (Gordon and Hen 2006; Heurteaux et al. 2006; Honoré 2007). Blocking TREK-1 and inhibiting the function of TREK-1 can have antidepressant effects (Djillani et al. 2019; Ye et al. 2015). TREK-1 knockout mice display a depression-resistant phenotype and show an increase in serotonin (5-HT) neurotransmission in the dorsal raphe nucleus (DRN) (Heurteaux et al. 2006). In agreement with the published findings, the results of this study demonstrate that the TREK-1 blocker alleviated depression-like behaviors in the CUS rats. The findings of this study and the above published reports confirm that TREK-1 is a molecular target for antidepressant action.

3.3 TREK-1 mediates the alleviative effects of the GABA_B R antagonist on depression-like behavior in chronic unpredictable stress rats

GABA_B R dysfunction is known to be involved in the pathogenesis of depression (Jacobson et al. 2018). It has been reported that TREK-1 plays a role in GABA_B R signaling in the hippocampus (Sandoz et al. 2012), and TREK-1 has antidepressant activity (Gordon and Hen 2006; Heurteaux et al. 2006; Honoré 2007). The results of this study showed that the GABA_B R antagonist alleviated depression-like behavior and reversed the decrease in TREK-1 protein expression in the hippocampus of the CUS rats; in contrast, the GABA_B R agonist exacerbated the above changes induced by CUS. In addition, a TREK-1 blocker (spadin) alleviated the depression-like behavior of the CUS rats but did not impact GABA_B R protein expression changes in the hippocampus of the CUS rats. These results demonstrate that activation or blockade of GABA_B R impacted depressive-like behavior via regulation of TREK-1 protein expression. The above results prove that TREK-1 mediated the alleviative effects of the GABA_B R antagonist on the depression-like behavior in chronic unpredictable stress rats. Notably, GABA_B R protein expression was significantly increased or decreased in the CUS rats treated with the GABA_B R antagonist or agonist, respectively, while TREK-1 protein expression was significantly increased in the CUS rats treated with the TREK-1 blocker. These results are due to the sensitization and desensitization of receptors after long-term drug intervention (Dale et al. 2014; Frank 2014). Compared with that in the untreated CUS rats, the expression of GABA_B R protein was upregulated by the GABA_B R antagonist and downregulated by the GABA_B R agonist. This is because long-term use of receptor antagonists will lead to an increase in the number of receptors, resulting in upregulation of receptors (Dudek et al. 2015); conversely, long-term use of receptor agonists will reduce the number of receptors, resulting in downregulation of receptors (Böhm et al. 1997). Moreover, the present results show that TREK-1 blockers can increase the expression of TREK-1 because long-term application of channel blockers will increase the number of channels and, through a negative feedback mechanism, increase the expression of channel proteins.

More importantly, the present results show that the GABA_B R antagonist and the TREK-1 blocker had the same antidepressant effect. In CUS rats, the GABA_B R antagonist increased TREK-1 expression, the GABA_B R agonist decreased TREK-1 expression, and the TREK-1 blocker had no effect on GABA_B R expression.

These results suggest that the GABA_B R may affect the expression of TREK-1 through downstream signaling molecules, but TREK-1 cannot impact GABA_B R protein expression (Fig. 3). These results suggest that the antidepressant effect of the GABA_B R is mainly mediated by TREK-1. TREK-1 affects the resting membrane potential by producing background leak-type potassium currents, thus improving the generation of bioelectricity in hippocampal neurons after stress stimulation and restoring their functions. Maintaining appropriate TREK-1 expression in the hippocampus may be the key cause of the antidepressant activity of GABA_B R antagonists. Therefore, this study proves not only that TREK-1 is involved in antidepressant activity as a downstream target of GABA_B R but also that GABA_B R antagonists could be applied to TREK-1 channel-related diseases.

The limitation is that the study does not provide evidence that the GABA_B R antagonist is ineffective in alleviating depression-like behavior in the absence of TREK-1 function or that the impact of the GABA_B R agonist on depression-like behavior can be suppressed by decreased TREK-1 function. In addition, other highly relevant brain regions, including the amygdala and frontal cortex, also need to be studied. We will continue to study the unclear mechanisms described above.

Conclusion

The novel findings in this study demonstrate that TREK-1 mediates the alleviative effects of GABA_B receptor antagonists on depression-like behavior in chronic unpredictable stress rats.

Declarations

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Authors' contributions

J.Y.L., H.R.L., and Z.Y.X. performed the experiments and drafted the manuscript. X.N.Z., X.LZ.. and W.Z.Y. performed data analysis. D.Q.Y., Y.G., H.X. and Y.X. acquired data and supervised the research. Y.S. and L.G. contributed new reagents or analytic tools. Z.Y.X., Y.Y.T., and S.M.Y. revised the manuscript. Z.Y.X. and S.M.Y. overall experimental guidance and paper writing guidance. Z.Y.X. was responsible for the conception and design of the present study and financial support. All authors read and approved the final manuscript.

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Data Availability

The data are not publicly available, but can be obtained from the corresponding authors according to reasonable requirements.

Compliance with Ethical Standards

Conflict of interest The authors declare no conflict of interest.

Ethics approval This work was permitted by Animal Experiments Ethical Committee of Dalian Medical University (AEE19087).

Consent for publication All authors agree to publish the manuscript in the journal.

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Figures

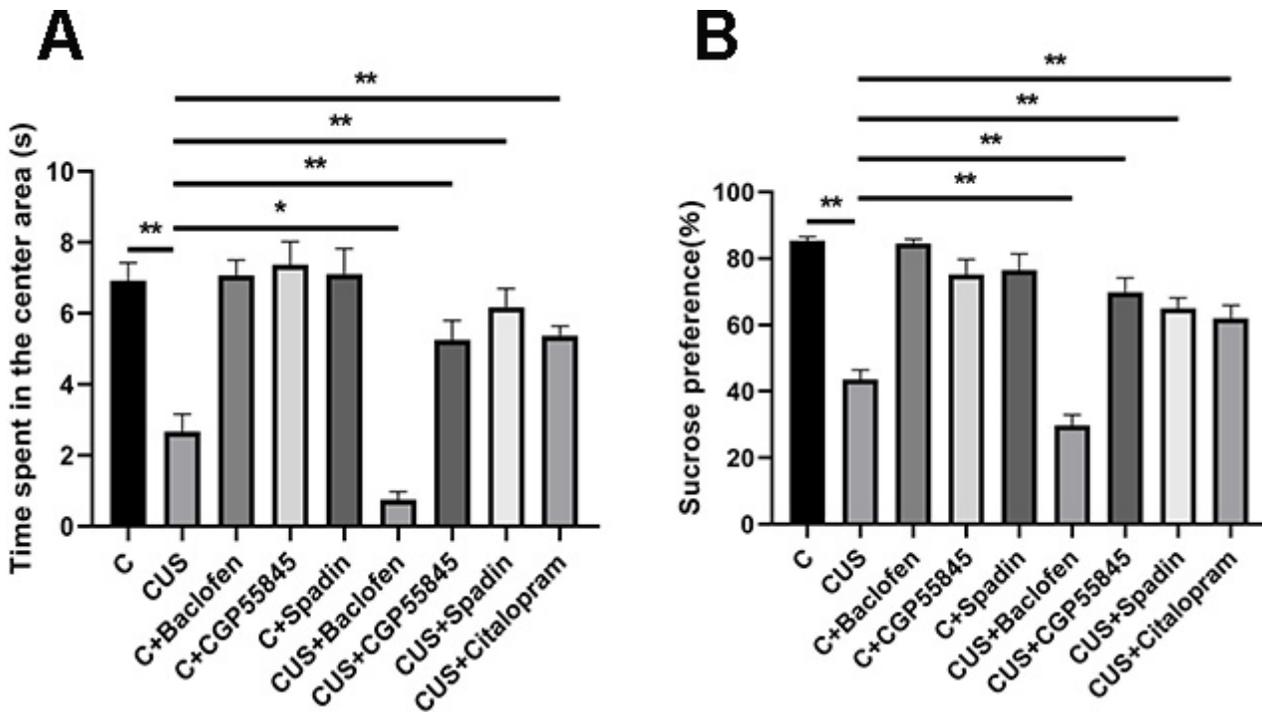


Figure 1

Both the GABAB R antagonist and the TREK-1 blocker alleviate depression-like behavior of the CUS rats. A. Time spent in central region; B. sucrose preference. Baclofen and CGP55845 are GABAB R agonist and antagonist respectively, spadin is TREK-1 blocker, citalopram is 5-HT reuptake inhibitor (positive control drug). The data are expressed as mean \pm standard deviation, * $p < 0.05$, ** $p < 0.01$ ($n=6-8$ rats/group).

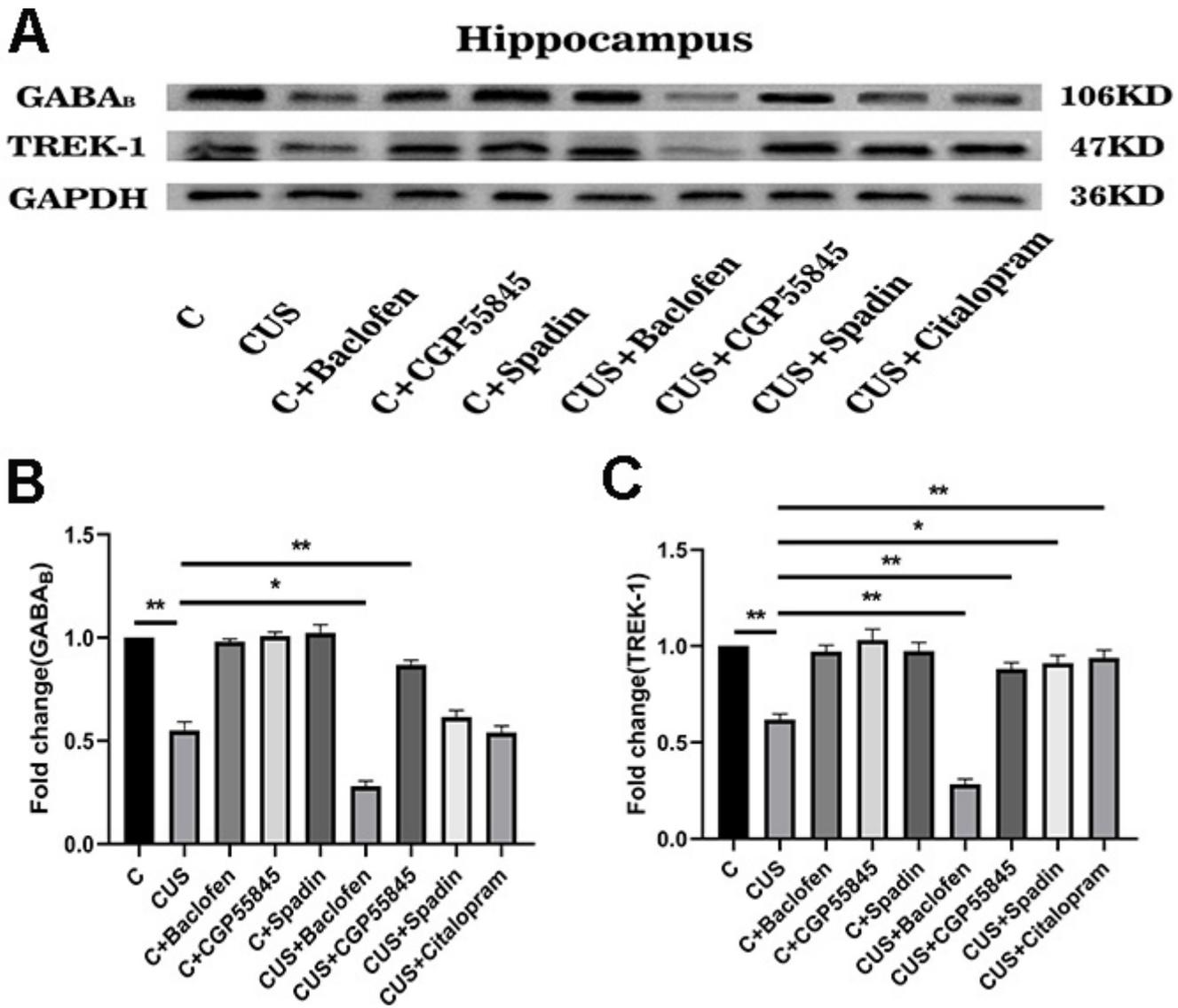


Figure 2

Both the GABA_B R antagonist and the TREK-1 blocker alleviate the low TREK-1 protein expression of the hippocampus in CUS rats. A. Western blot results of TREK-1 and GABA_B R in the hippocampus; B. Analysis results of GABA_B R protein expression; C. Analysis results of TREK-1 protein expression. Baclofen and CGP55845 are GABA_B R agonist and antagonist, spadin is TREK-1 blocker, citalopram is 5-HT reuptake inhibitor (positive control drug). The data are expressed as mean \pm standard deviation, * $p < 0.05$, ** $p < 0.01$ (n=5 rats/group).

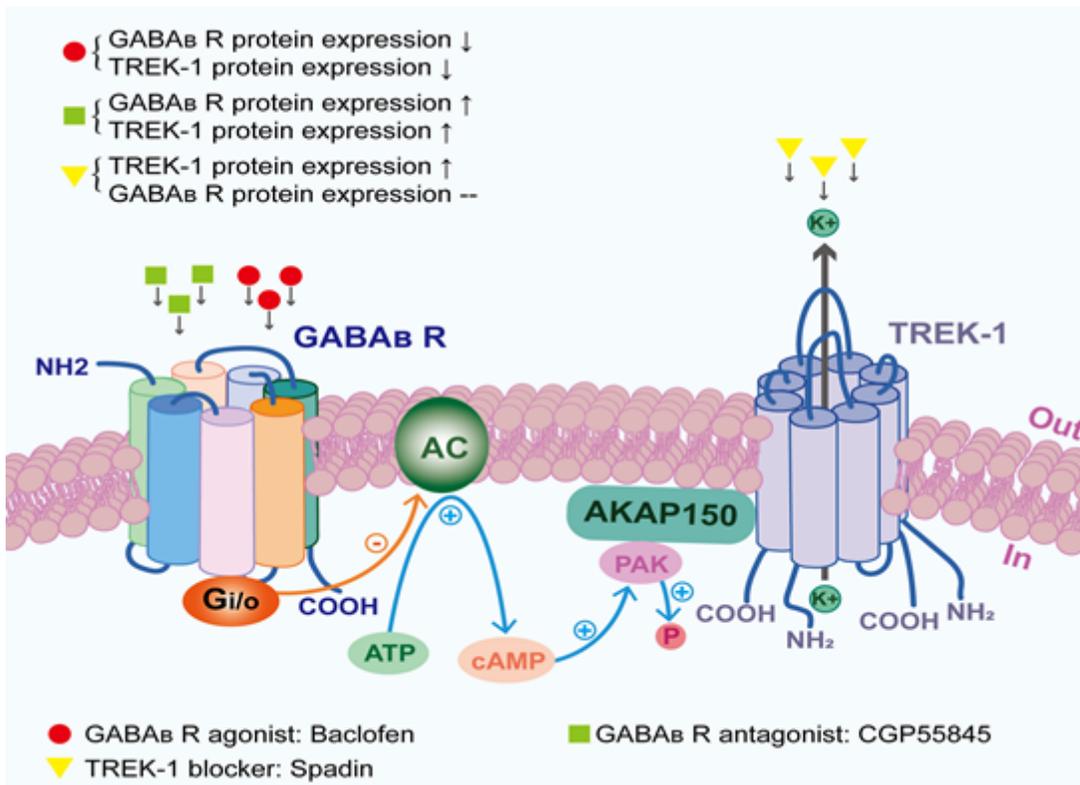


Figure 3

The effect of GABA_B receptor (GABA_B R) on the expression of TREK-1 through downstream signaling molecules. Baclofen (GABA_B R agonist) activates GABA_B R, resulting in inhibiting Gi/O G-protein activation. Gi/O G-protein activation inhibits the activity of adenylate cyclase (AC), thereby reducing the conversion of ATP to cyclic adenosine monophosphate (cAMP), and inhibiting the activation of protein kinase A (PKA) (Asaoka et al. 2017). PKA needs kinase A anchored proteins (AKAPs) to bind PKA to TREK-1 channel (Deng et al. 2009). GABA_B R mediates PKA inhibition, thereby enhancing the function of TREK-1 channel, and ultimately inhibiting the excitability of hippocampal neurons. GABA_B R antagonist increases TREK-1 expression, GABA_B R agonist decreases TREK-1 expression, and TREK-1 blocker has no effect on GABA_B R expression.