Alcohol and Alcoholism, 2022, 57(2) 234–241
https://doi.org/10.1093/alcalc/agab065
Advance Access Publication Date: 22 September 2021
Article

OXFORD

Article

Gabapentin Reduces Alcohol Intake in Rats by Regulating NF-κB Signaling Pathway Via PPAR γ

Jing Li, Kewei Xu, Hao Ding, and Qiaozhen Xi*

Rehabilitation Department, Qingdao Mental Health Center, Shandong 266034, PR China

*Corresponding author: Qingdao Mental Health Center, NO 299, Nanjing Road, Shandong 266034, P.R. China; E-mail: xqzqd@hotmail.com

Received 23 May 2021; Revised 21 July 2021; Editorial Decision 26 August 2021; Accepted 26 August 2021

Abstract

Aims: Increasing preclinical and clinical reports have demonstrated the efficacy of gabapentin (GBP) in treating alcohol use disorder (AUD). However, the mechanism of the effects of GBP in AUD is largely unknown. Herein, we sought to investigate the effect of GBP in a rat model of AUD and explore the underlying mechanism.
Methods: The intermittent access to 20% ethanol in a 2-bottle choice (IA2BC) procedure was exploited to induce high voluntary ethanol consumption in rats. The rats were treated daily for 20 days with different doses of GBP, simultaneously recording ethanol/water intake. The locomotor activity and grooming behavior of rats were also tested to evaluate the potential effects of GBP on confounding motor in rats. The levels of IL-1β and TNF-α in serum and hippocampus homogenate from the rats were detected by using ELISA. The expressions of peroxisome proliferator-activated-receptor γ (PPAR-γ) and nuclear factor-κB (NF-κB) in the hippocampus were determined by immunofluorescence and western blot.
Results: GBP reduced alcohol consumption, whereas increased water consumption and locomotor activity of rats. GBP was also able to decrease the levels of IL-1β and TNF-α in both serum and hippocampus, in addition to the expression of NF-κB in the hippocampus. Furthermore, these effects attributed to GBP were observed to disappear in the presence of bisphenol A diglycidyl ether (BADGE), a specific inhibitor of PPAR-γ.
Conclusions: Our findings revealed that GBP could activate PPAR-γ to suppress the NF-κB signaling pathway, contributing to the decrease of ethanol consumption and ethanol-induced neuroimmune responses.

INTRODUCTION

Long-term and excessive alcohol misuse might develop into an alcohol use disorder (AUD), thereby inducing various diseases including liver cirrhosis, cardiovascular disease and cancer. However, only a few medications are approved by the FDA for AUD therapy nowadays (Knox et al., 2019). Moreover, these approved drugs have limited potency for the long-term management of AUD and exhibit serious adverse effects (Witkiewitz et al., 2019). Thus, there is an increasing demand to develop new strategies against AUD.

As an anticonvulsant approved by FDA, gabapentin (GBP) is a structural analog of the neurotransmitter γ-aminobutyric acid (GABA), which has been proven to be clinically effective for AUD in recent years (Anton et al., 2020). Notably, GBP has no effect on GABA uptake or degradation as it does not bind to GABA receptors, but indirectly influences GABA by binding with high affinity to the $\alpha 2\delta$-1 site of voltage-sensitive calcium channels (Suto et al., 2014, Zamponi et al., 2015). Nonetheless, the underlying mechanism of GBP in improving AUD remains undefined. As known, endogenous

© The Author(s) 2021. Medical Council on Alcohol and Oxford University Press. All rights reserved.

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

opioid systems are crucial in the reinforcement and motivational aspects of substance abuse (Trigo et al., 2010). Bannister et al. (2017) indicated that GBP induced dopamine release in the rostral anterior cingulate cortex via an endogenous opioid signaling pathway to reduce neuropathic pain. Increasing research demonstrated that the neuroimmune system, especially innate immune responses in the peripheral and central nervous systems, is a vital target of AUD that may contribute to abuse and dependence (Mayfield et al., 2013). The identification of neuroimmune activation in AUD has been supported by findings that the persistent upregulation of key immune molecules was found in postmortem human alcoholic brain tissue and rodents (Crews and Vetreno, 2014). *In vitro* studies also found that ethanol can cause activation in both microglial cells and astrocyte cells, leading to the induction of proinflammatory cytokines (TNFα and IL-1β) (Alfonso-Loeches et al., 2010, Qin and Crews, 2012). *Ex vivo* brain slice cultures also exhibited immune activation with ethanol exposure (Zou and Crews, 2010), further supporting that ethanol can directly cause neuroimmune activation, in the absence of peripheral immune involvement. Thus, targeted and strategic neuroimmune therapies might be promising in the treatment of patients with AUD. It has been documented that peroxisome proliferator-activated receptor γ (PPAR-γ) is highly expressed in glial cells and neurons where it exerts effects of neuroprotection, cell repair and anti-inflammatory responses (Garrido-Gil et al., 2012, Villapol, 2018). Some evidence suggested that the activation of brain PPAR-γ receptors exhibited a potential for AUD treatments (Fotio et al., 2021). Recently, de Brito et al. (2020) revealed that GBP could activate the PPAR-γ receptor to play a suppressive role on nuclear factor-κB (NF-κB) signaling, thereby reducing the activation of inflammatory genes related to inflammatory bowel diseases.

This study aims to investigate the GBP for its effects on the alcohol-consuming rat model; and we hypothesize that GBP plays the 'anti-alcohol' effect by activating PPAR-γ, which in turn suppresses the NF-κB signaling pathway.

MATERIALS AND METHODS

Animals

Male Sprague–Dawley (SD) rats (SPF grade, 8–10 weeks old, 220–260 g) were purchased from Qingdao Institute of Drug Control (Qingdao, China). The total number of rats used in this study was 70. All rats in the current study were housed in a temperature- and humidity-controlled condition under a regular 12-hour light–dark cycle (lights off at 7:30 PM) with a chow diet and sterile water available *ad libitum*. After the beginning of treatments, the alcohol consumption and water intake of each rat were calculated and described as g/kg/day and mL/kg/day, respectively.

All the experimental protocols were reviewed and approved by our Academy Animal Experimental Ethical Committee. All procedures were conducted in adherence to the requirements of the General Recommendations of Chinese Experimental Animals.

Establishment of intermittent access to 20% ethanol in a 2-bottle choice model

Based on a two-bottle choice ethanol consumption procedure, intermittent access to ethanol was carried out as previously described (Carnicella et al., 2014). After training for 4 weeks, the intermittent access to 20% ethanol in a 2-bottle choice (IA2BC) rat model was successfully established, which was characterized by stably high levels of ethanol consumption (>5.5 g/kg/day). After 4 weeks of alcohol ingestion, the blood ethanol concentration of the IA2BC rats was 36.3 ± 10.3 mg/%. Additionally, rats showed obvious alcohol withdrawal symptoms, including stereotyped behavior, irritability, stiff tail and abnormal gait, after withdrawal of alcohol.

Experimental design of the treatment

The schematic representation of this experiment is presented in Figure 1A. After successfully establishing the IA2BC model rat, 40 rats were maintained under the 24 h continuous access two-bottle free-choice paradigm for further 21 days, and received different treatment ($N = 10$ per group) according to experimental design follows: GBP-L group (30 mg/kg), GBP-M group (60 mg/kg) and GBP-H group (120 mg/kg). The saline group rats treated with saline were considered as control. GBP or saline was administrated once daily for 20 days. The concentrations of GBP were selected based on previous studies (McDonald et al., 2008, Besheer et al., 2016).

The remaining 30 rats were used to investigate if the activation of PPAR-γ contributes to the effect of GBP in reducing the alcohol consumption of chronically alcoholic rats. In this study, the activation of PPAR-γ was blocked by using bisphenol A diglycidyl ether (BADGE; a PPAR-γ inhibitor). The corresponding experimental design was as follows: (a) Saline group, (b) GBP group (60 mg/kg GBP) and (c) GBP+ BADGE group (60 mg/kg GBP + 30 mg/kg BADGE). The GBP and BADGE were respectively administered by oral gavage and intraperitoneal injection.

Examination of locomotor activity

Locomotor activity was examined by using the open-field test, in accordance with the protocol of a previous study (Rivera-Meza et al., 2014). The rats with different treatments were respectively placed in the center of the open-field apparatus, and their locomotor activity was recorded by the automated system for 30 min. We defined an activity unit (AU) as a complete crossing from one square to another; and locomotor activity was represented as AUs/5 min. After each examination, the apparatus was cleaned with water and dried using paper towels. The time (s) spent on grooming behavior was also recorded.

Sample collection

Rats were anesthetized with sodium pentobarbital solution. After collecting blood from the lateral tail vein (0.2 ml per rat), rats were sacrificed by decapitation for immediate brain sampling within 2 h of cessation of alcohol consumption. The serum was acquired by centrifuging collected blood. A recent study revealed that the hippocampus is the main distribution of chronic alcohol-induced peripheral macrophage infiltration (neuroinflammation; Lowe et al., 2020). Additionally, the hippocampus is one of the target regions of GBP in several studies; so, the hippocampus was carefully isolated from brain tissues on ice for further analysis. One-half of hippocampus tissues were fixed in 4% paraformaldehyde solution for subsequent experiments. The remaining sample was mixed with saline solution and homogenized in an ice bath by a glass homogenizer to obtain hippocampal homogenate. After centrifugation of the hippocampal homogenates, supernatants were collected and immediately stored at −80°C.

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

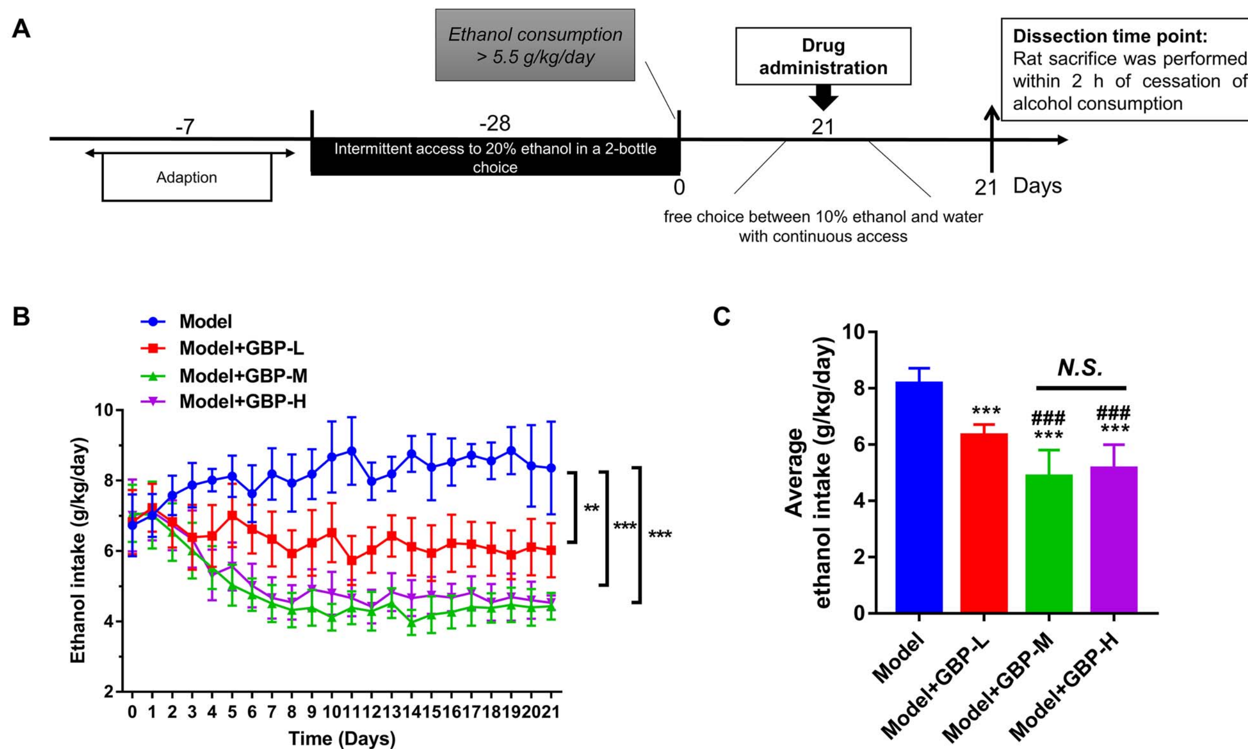

Fig. 1. GBP reduces the ethanol consumption of IA2BC model rats. (A) Timeline, schematic representation of the experimental procedure. **(B)** Influence of 20 days of GBP treatment on the ethanol intake of IA2BC model rats. **(C)** Average ethanol intake of IA2BC model rats during 20 days treatment. Note: $^{**}P < 0.01$, $^{***}P < 0.005$ versus saline group; $^{\#\#\#}P < 0.005$ versus GBP-L group.

Enzyme linked immunosorbent assays

The levels of IL-1β and TNF-α in serum and hippocampus homogenate were detected by using enzyme linked immunosorbent assays (ELISA) kits (BMS630 and BMS622; Thermo Fisher), in accordance with the manufacturer's manual. Absorbance was spectrophotometrically recorded at both 450 nm and 630 nm.

Immunofluorescence staining

Hippocampus sections from saline and GBP group ($n = 5$, per group) were rinsed twice and incubated with 0.1 M PBS containing 0.1% Triton X-100 and 2% normal goat/rabbit serum for an hour. The sections were incubated with primary antibodies (PPAR-γ and NF-κB/p65) at 4°C overnight, followed by incubated with TRITC and FITC-labeled secondary antibodies for 90 min. The sections were mounted and incubated with DAPI, before observing under a fluorescence microscope.

Western blot

Total protein of hippocampus ($n = 5$, per group) was extracted using RIPA lysate, of which concentration was determined using a BCA Protein Assay kit. Protein (50 μg) was separated by SDS-PAGE gel, then transferred onto PVDF membranes. The membranes were blocked, and then incubated with primary antibodies (GAPDH, PPAR-γ, and NF-κB/p65) overnight. Afterwards, the membranes were rinsed thrice and subsequently incubated with an HRP-conjugated secondary antibody. Blots were detected and visualized with ECL reagent (WBKLS0050; EMD Millipore).

Statistics

All data are expressed as the mean ± SEM. Statistical analysis was conducted based on GraphPad Prism software version 8.0.1. The comparisons between the alcohol/water intake of GBP- and saline-treated rats were analyzed using two-way ANOVA followed by Tukey's post hoc test. Data of the other experiments were analyzed using two-tailed Student's t-test or one-way ANOVA followed by Bonferroni's post hoc test. In this study, $P < 0.05$ was considered to represent a statistically significant discrepancy.

RESULTS

GBP reduces the ethanol consumption of IA2BC model rats

To investigate the effect of different doses of GBP on the maintenance of voluntary ethanol intake, IA2BC model rats were given a free choice between 10% ethanol (v/v) and water for 3 weeks. On the first day of ethanol access, rats were administered high, medium, and low dose of GBP or saline each day for 20 consecutive days. As shown in Figure 1B, all doses of GBP significantly decreased the ethanol intake of rats compared to saline (interaction [F (63, 440) = 5.899, $P < 0.0001$]; days [F (21, 440) = 8.75, $P < 0.0001$]; doses [F (3, 440) = 631.3, $P < 0.0001$]). In the meantime, compared with the treatment of saline, the treatment of GBP could remarkably reduce the average ethanol intake of rats ([F (3, 80) = 91.13, $P < 0.0001$]; Fig. 1C). As we can see, both the effect of medium and high doses of GBP in reducing the average ethanol intake were significantly stronger than that of low dose of GBP ($P < 0.005$), but there was no statistical difference in the comparison between GBP groups in

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

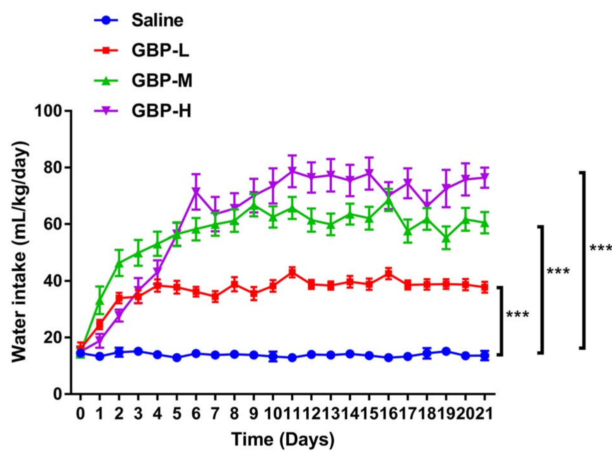

Fig. 2. GBP increases the water intake of IA2BC model rats. Note: *** $P < 0.005$ versus saline group.

either medium or high doses. Therefore, the medium dose of GBP was selected to perform the subsequent experiments.

Additionally, in comparison to saline, all doses of GBP significantly increased the water intake of rats (interaction [F (63, 440) = 42.84, $P < 0.0001$]; days [F (21, 440) = 165.5, $P < 0.0001$]; doses [F (3, 440) = 5266, $P < 0.0001$]; Fig. 2).

GBP enhances the locomotor activity, and reduces proinflammatory cytokines level of IA2BC model rats

In order to explore whether the GBP could also affect locomotor activity and proinflammatory cytokines biosynthesis of IA2BC model rats, the locomotor activity, as well as IL-1β and TNF-α levels of GBP- and saline-treated rats were evaluated. For these experiments, rats were administered a medium dose of GBP used in the formerly mentioned experiments. As shown in Figure 3A, the locomotor activity of rats treated with GBP was higher than that of rats treated with saline (treatment [F (1, 50) = 14.87, $P = 0.0003$]). However, GBP treatment did not significantly affect the grooming activity of IA2BC model rats (Fig. 3B, treatment [F (1, 50) = 0.02814, $P = 0.8674$])).

The level of IL-1β and TNF-α were detected in both the serum and hippocampus of rats by performing ELISA. The results showed that both IL-1β and TNF-α serum levels were reduced by GBP treatment (Fig. 3C). Similarly, both IL-1β and TNF-α levels in the hippocampus of the GBP group were also significantly lower than those of the saline group (Fig. 3D). These data suggested that the treatment of GBP alleviated inflammation induced by alcohol consumption.

GBP increases the expression of NF-κB and PPAR-γ in the hippocampus from IA2BC model rats

Given the crucial role of NF-κB in neuroimmune and the neuroprotective effect of PPAR-γ in the brain (Garrido-Gil et al., 2012,

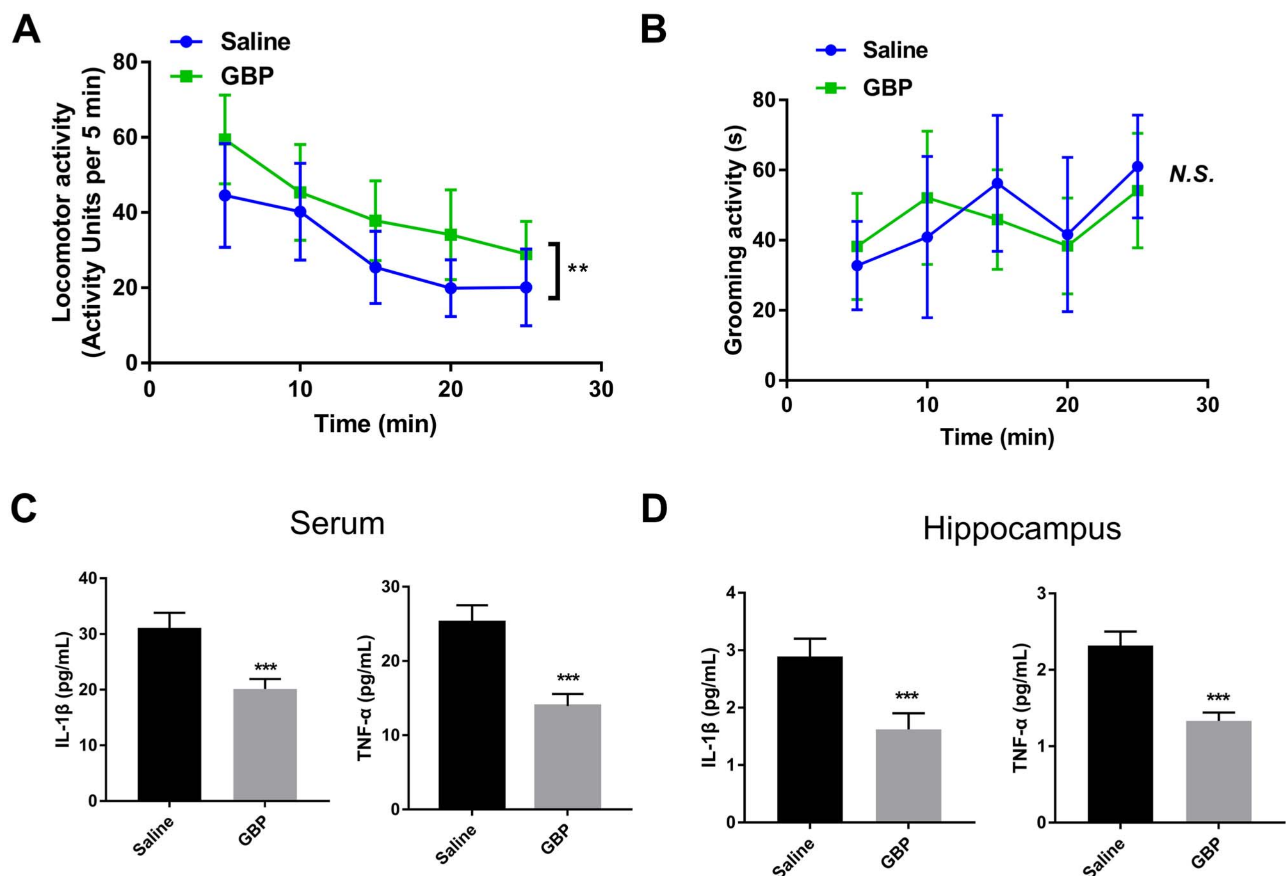

Fig. 3. GBP reduces the locomotor activity, as well as proinflammatory cytokines level of IA2BC model rats. The effect of GBP on **(A)** locomotor activity and **(B)** grooming activity. The level of IL-1β and TNF-α were detected in both **(C)** serum and **(D)** hippocampus of IA2BC model rats. Note: ** $P < 0.01$, *** $P < 0.005$ versus saline group.

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

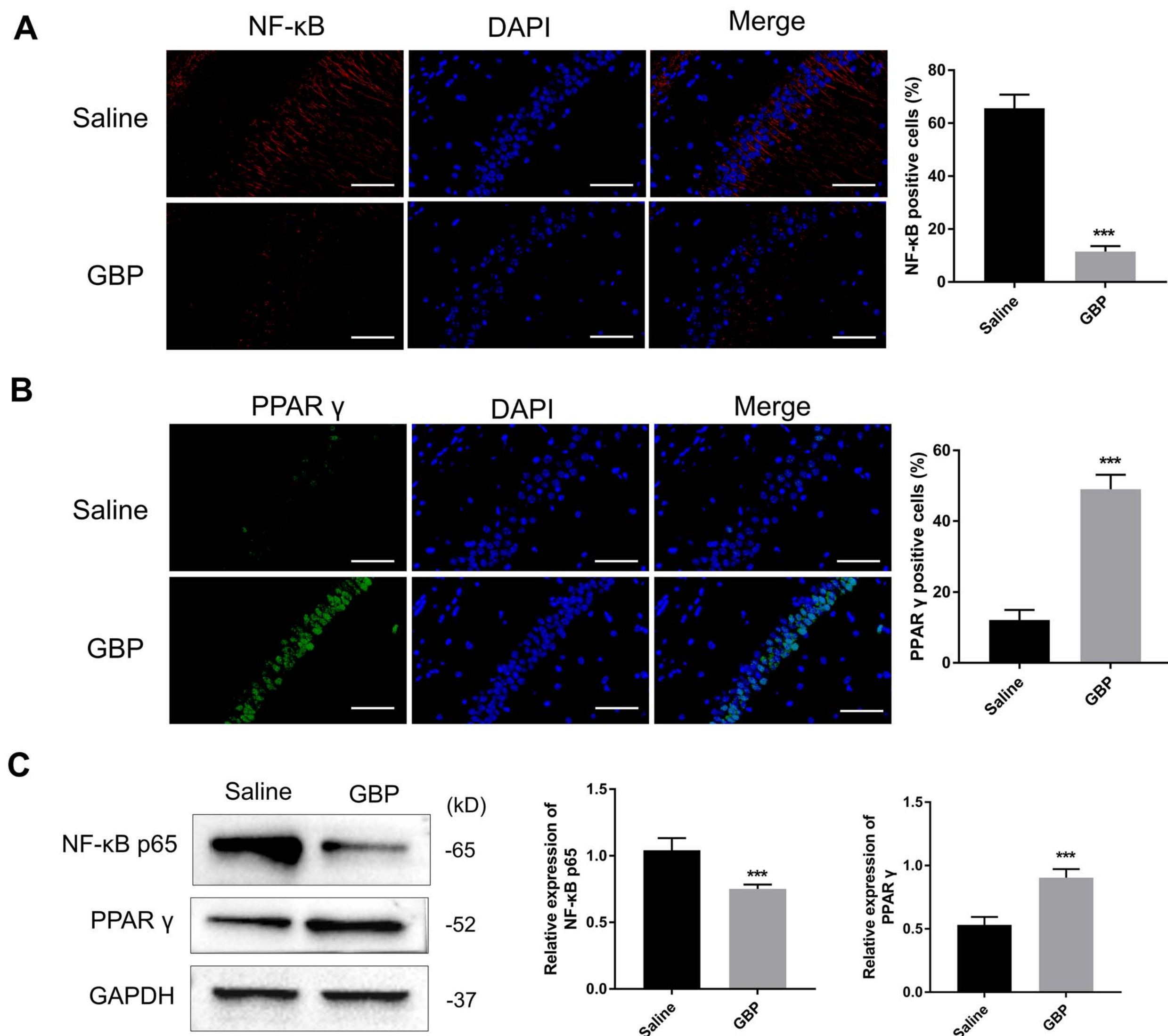

Fig. 4. GBP increases the expression of NF-κB and PPAR-γ in the hippocampus from IA2BC model rats. Representative immunofluorescence images (×400) of hippocampus demonstrating protein expression of **(A)** NF-κB and **(B)** PPAR-γ. Scale bar = 50 μm. **(C)** The protein expression of NF-κB and PPAR-γ detected by western blot. Note: *** $P < 0.005$ versus saline group.

Nennig and Schank, 2017), we detected the expression of NF-κB and PPAR-γ in the hippocampus of rats. We found that the expression of NF-κB in the GBP group was obviously lower than that in the saline group (Fig. 4A). Conversely, the expression of PPAR-γ was significantly increased in the GBP group relative to the saline group (Fig. 4B). Consistent with the immunofluorescence assay results, a large quantity of NF-κB protein was detected by western blot in the saline group (Fig. 4C). However, the GBP group demonstrated significantly lower levels of NF-κB relative to the saline group. Western blot results also revealed that the PPAR-γ protein level of the GBP group was significantly elevated in comparison of the saline group (Fig. 4C). These findings suggested the involvement of NF-κB and PPAR-γ in the effect of GBP on AUD.

GBP reduces the alcohol consumption of IA2BC model rats by regulating the NF-κB signaling pathway via PPAR-γ

To further verify whether the effect of GBP on AUD is exerted through NF-κB signaling pathway via the upregulation of PPAR-γ, we blocked the activation of PPAR-γ by BADGE, a specific PPAR-γ inhibitor. As shown in Figure 5A, the average ethanol intake of rats in the GBP group (4.8 ± 0.8) was significantly lower than that in the saline group (8.4 ± 0.9). However, when the GBP group was cotreated with BADGE, a significant increase in average ethanol intake (7.0 ± 0.8) was observed. Additionally, GBP treatment led to an observable promotion of the locomotor activity of IA2BC model rats; this effect was slightly prohibited by BADGE (not significant; Fig. 5B). Results of ELISA demonstrated that rats of the GBP group

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

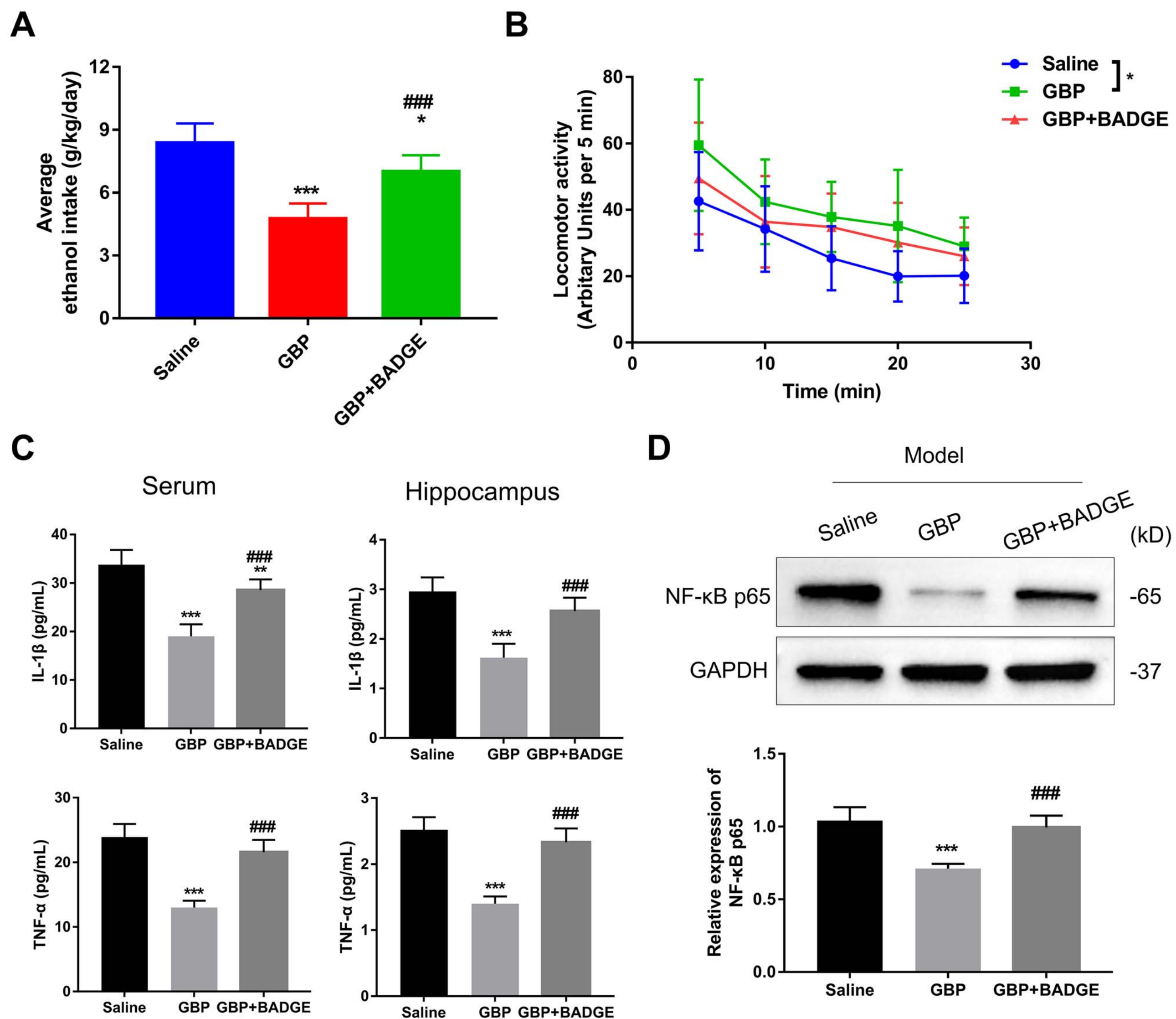

Fig. 5. GBP reduces the alcohol consumption of IA2BC model rats by regulating NF-κB signaling pathway via PPAR-γ. (A) Average reduction in ethanol intake and **(B)** locomotor activity of IA2BC model rats after the treatment of saline, GBP, or GBP + BADGE. **(C)** The level of IL-1β and TNF-α were detected in both serum and hippocampus of IA2BC model rats. **(D)** The protein expression of NF-κB in the hippocampus of IA2BC model rats detected by western blot. Note: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ versus saline group; $^{###}P < 0.005$ versus GBP group.

exhibited significantly lower proinflammatory cytokines levels than rats of the saline group. Moreover, BADGE treatment was observed to reverse the effect of GBP in reducing proinflammatory cytokines generation of IA2BC model rats (Fig. 5C). Consistent with the former experiment, GBP treatment significantly increased the expression of NF-κB compared to saline treatment. As expected, this effect was also countered by BADGE (Fig. 5D).

DISCUSSION

A growing amount of evidence supports the safety and efficacy of GBP as a novel option in AUD therapy, with unique advantages for alcohol-related negative effects and insomnia, in comparison to available treatments (Mason et al., 2009, Falk et al., 2019). Although promising, the molecular mechanisms by which GBP reduces AUD symptomatology must be uncovered to facilitate the progression of GBP into the clinic for AUD. In this study, we found that GBP exerts a suppressing effect on alcohol-related behaviors, and this effect is mediated by activating PPAR-γ receptors.

Initially, we established an ethanol-dependent model using the IA2BC drinking paradigm in SD rats. Our study indicated that the administration of GBP effectively reduces the alcohol intake of IA2BC rats. All concentrations of GBP resulted in an obvious decrease in voluntary ethanol consumption and an attendant increase in water intake. Our data showed that the effect of GBP appeared from the first day after administration and was maintained for the entire treatment period. Notably, the action of GBP in reducing alcohol intake obviously enhanced from 30 mg/kg to 60 mg/kg, but slightly reduced from 60 mg/kg to 120 mg/kg. This reduction of effectiveness in high dose GBP may be due to the development of tolerance. Sotomayor-Zárate et al. (2013) have also observed this situation in UChB rats with cytisine and varenicline. It is widely accepted that ethanol can depress locomotion in rats. Our study revealed that IA2BC rats treated with GBP showed an enhanced locomotor activity

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

compared to that shown by control rats. We supposed that it may be because GBP decreased the ethanol intake of rats, thereby impairing the inhibitory effect induced by ethanol on locomotor activity in rats.

During the development of AUD, alcohol and its metabolites induce oxidative stress and increasing systemic endotoxins, thereby provoking immune cells to release proinflammatory cytokines in the periphery followed by triggering brain microglia and astrocytes to secrete cytokines of the central nervous system (Neupane, 2016). Therefore, AUD is now considered a systemic inflammatory condition, and cytokines can be used as biomarkers of alcohol abuse (Achur et al., 2010). Mayfield et al. (2013) revealed that cytokines play an important role in the voluntary alcohol intake of rodents. Notably, a previous shown that alcohol did not affect mRNA levels of some cytokines in the hippocampus, cerebellum, or cortex of adolescent mice, as well as the cerebellum of adult mice, suggesting an age- and region-specific susceptibility to ethanol regulation of neuroinflammatory and addiction-related molecules (Kane et al., 2014). Based on these, we detected proinflammatory cytokines levels to determine whether GBP reduces AUD-related symptoms by attenuating neuroinflammation. As expected, the levels of TNF-α and IL-1β in both serum and hippocampus of IA2BC rats were significantly reduced by GBP treatment. Increasing evidence has implicated that high-alcohol consumption results in activating the NF-κB signaling pathway (Blednov et al., 2012, Cantacorps et al., 2020). NF-κB translocated from the cytoplasm to the nucleus and bound to DNA, which resulted in the secretion of proinflammatory cytokines, such as TNF-α and IL-1β. A previous study reported that PPAR-γ activation induces anti-inflammatory effects through the inhibition of NF-κB (Wang et al., 2016). Additionally, de Brito et al. (2020) demonstrated that GBP exerted its anti-inflammatory effect by interacting with PPAR-γ. This lets us suppose the possibility that the effect of GBP on ethanol-induced neuroinflammation may also be dependent on the activation of PPAR-γ. Both immunofluorescence and western blot showed that GBP treatment significantly reduced the expression of NF-κB in the hippocampus. Meanwhile, GBP treatment also led to a significant elevation of the expression of PPAR-γ in hippocampus. These findings suggested that GBP reduces major cytokines involved in ethanol-induced neuroinflammation (IL-1β and TNF-α) by suppressing the NF-κB pathway via PPAR-γ activation. In order to further confirm the above findings, IA2BC rats were cotreated with GBP and BADGE. In the presence of BADGE, GBP lost its actions on alcohol consumption and locomotor activity of IA2BC rats. Besides, the inhibition of GBP in decreasing the expression of IL-1β, TNF-α and NF-κB was also reversed by BADGE. Collectively, these data indicated that the effect of GBP on the amelioration of AUD seems to be dependent on PPAR-γ activation.

In conclusion, our study confirmed the effect of GBP in the treatment of AUD, and preliminarily uncover the underlying mechanism. By activating that PPAR-γ, GBP down-regulated the NF-κB signaling pathway to reduce the secretion of proinflammatory cytokines, rendering to the decrease of alcohol consumption of IA2BC model. This study gave further support to the idea that GBP is an effective drug for the treatment of AUD, and provided a better understanding of the mechanism of GBP in AUD.

DATA AVAILABILITY

The data supporting the findings in this study are included within the article.

FUNDING

The authors received no specific funding for this study.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

Achur RN, Freeman WM, Vrana KE. (2010) Circulating cytokines as biomarkers of alcohol abuse and alcoholism. *J Neuroimmune Pharmacol* 5: 83–91.

Alfonso-Loeches S, Pascual-Lucas M, Blanco AM *et al.* (2010) Pivotal role of TLR4 receptors in alcohol-induced neuroinflammation and brain damage. *J Neurosci* 30:8285–95.

Anton RF, Latham P, Voronin K *et al.* (2020) Efficacy of gabapentin for the treatment of alcohol use disorder in patients with alcohol withdrawal symptoms: a randomized clinical trial. *JAMA Intern Med* 180:728–36.

Bannister K, Qu C, Navratilova E *et al.* (2017) Multiple sites and actions of gabapentin-induced relief of ongoing experimental neuropathic pain. *Pain* 158:2386–95.

Besheer J, Frisbee S, Randall PA *et al.* (2016) Gabapentin potentiates sensitivity to the interoceptive effects of alcohol and increases alcohol self-administration in rats. *Neuropharmacology* 101:216–24.

Blednov YA, Ponomarev I, Geil C *et al.* (2012) Neuroimmune regulation of alcohol consumption: behavioral validation of genes obtained from genomic studies. *Addict Biol* 17:108–20.

Cantacorps L, Montagud-Romero S, Valverde O. (2020) Curcumin treatment attenuates alcohol-induced alterations in a mouse model of foetal alcohol spectrum disorders. *Prog Neuropsychopharmacol Biol Psychiatry* 100:109899.

Carnicella S, Ron D, Barak S. (2014) Intermittent ethanol access schedule in rats as a preclinical model of alcohol abuse. *Alcohol* 48:243–52.

Crews FT, Vetreno RP. (2014) Neuroimmune basis of alcoholic brain damage. *Int Rev Neurobiol* 118:315–57.

DE Brito TV, Júnior GJD, Da Cruz Júnior JS *et al.* (2020) Gabapentin attenuates intestinal inflammation: role of PPAR-gamma receptor. *Eur J Pharmacol* 873:172974.

Falk DE, Ryan ML, Fertig JB *et al.* (2019) Gabapentin Enacarbil extended-release for alcohol use disorder: a randomized, double-blind, placebo-controlled, multisite trial assessing efficacy and safety. *Alcohol Clin Exp Res* 43:158–69.

Fotio Y, Borruto AM, Benvenuti F *et al.* (2021) Activation of peroxisome proliferator-activated receptor γ reduces alcohol drinking and seeking by modulating multiple mesocorticolimbic regions in rats. *Neuropsychopharmacology* 46:360–7.

Garrido-Gil P, Joglar B, Rodriguez-Perez AI *et al.* (2012) Involvement of PPAR-γ in the neuroprotective and anti-inflammatory effects of angiotensin type 1 receptor inhibition: effects of the receptor antagonist telmisartan and receptor deletion in a mouse MPTP model of Parkinson's disease. *J Neuroinflammation* 9:38.

Kane CJ, Phelan KD, Douglas JC *et al.* (2014) Effects of ethanol on immune response in the brain: region-specific changes in adolescent versus adult mice. *Alcohol Clin Exp Res* 38:384–91.

Knox J, Hasin DS, Larson FRR *et al.* (2019) Prevention, screening, and treatment for heavy drinking and alcohol use disorder. *Lancet Psychiatry* 6:1054–67.

Lowe PP, Morel C, Ambade A *et al.* (2020) Chronic alcohol-induced neuroinflammation involves CCR2/5-dependent peripheral macrophage infiltration and microglia alterations. *J Neuroinflammation* 17:296.

Mason BJ, Light JM, Williams LD *et al.* (2009) Proof-of-concept human laboratory study for protracted abstinence in alcohol dependence: effects of gabapentin. *Addict Biol* 14:73–83.

Mayfield J, Ferguson L, Harris RA. (2013) Neuroimmune signaling: a key component of alcohol abuse. *Curr Opin Neurobiol* 23:513–20.

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022

Mcdonald LM, Sheppard WF, Staveley SM *et al.* (2008) Discriminative stimulus effects of tiagabine and related GABAergic drugs in rats. *Psychopharmacology (Berl)* 197:591–600.

Nennig SE, Schank JR. (2017) The role of NFkB in drug addiction: beyond inflammation. *Alcohol Alcohol* 52:172–9.

Neupane SP. (2016) Neuroimmune Interface in the comorbidity between alcohol use disorder and major depression. *Front Immunol* 7:655.

Qin L, Crews FT. (2012) NADPH oxidase and reactive oxygen species contribute to alcohol-induced microglial activation and neurodegeneration. *J Neuroinflammation* 9:5.

Rivera-Meza M, Quintanilla ME, Bustamante D *et al.* (2014) Overexpression of hyperpolarization-activated cyclic nucleotide-gated channels into the ventral tegmental area increases the rewarding effects of ethanol in UChB drinking rats. *Alcohol Clin Exp Res* 38:911–20.

Sotomayor-Zárate R, Gysling K, Busto UE *et al.* (2013) Varenicline and cytisine: two nicotinic acetylcholine receptor ligands reduce ethanol intake in University of Chile bibulous rats. *Psychopharmacology (Berl)* 227:287–98.

Suto T, Severino AL, Eisenach JC *et al.* (2014) Gabapentin increases extracellular glutamatergic level in the locus coeruleus via astroglial glutamate transporter-dependent mechanisms. *Neuropharmacology* 81:95–100.

Trigo JM, Martin-García E, Berrendero F *et al.* (2010) The endogenous opioid system: a common substrate in drug addiction. *Drug Alcohol Depend* 108:183–94.

Villapol S. (2018) Roles of peroxisome proliferator-activated receptor gamma on brain and peripheral inflammation. *Cell Mol Neurobiol* 38:121–32.

Wang X, Sun Y, Zhao Y *et al.* (2016) Oroxyloside prevents dextran sulfate sodium-induced experimental colitis in mice by inhibiting NF-κB pathway through PPARγ activation. *Biochem Pharmacol* 106:70–81.

Witkiewitz K, Litten RZ, Leggio L. (2019) Advances in the science and treatment of alcohol use disorder. *Sci Adv* 5:eaax4043.

Zamponi GW, Striessnig J, Koschak A *et al.* (2015) The physiology, pathology, and pharmacology of voltage-gated calcium channels and their future therapeutic potential. *Pharmacol Rev* 67:821–70.

Zou J, Crews F. (2010) Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF-κB and proinflammatory cytokines. *Alcohol Clin Exp Res* 34:777–89.

Downloaded from https://academic.oup.com/alcalc/article/57/2/234/6374132 by University of San Diego user on 28 December 2022