

Modifications in Gamma-aminobutyric Acid type A Receptor Subunit Gene Expression During Macrophage Differentiation and Propofol Administration in THP-1 Cells

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Abstract

Background: Gamma-aminobutyric acid type A (GABA_A) receptors are thought to play a role in the functioning of the immune system. GABA_A receptors have 19 types of subunits, the components of which determine their physiological functions. However, the subunits that are expressed in immune cells during inflammation have not been fully investigated. Recent reports have shown that anesthetic agents may affect the gene expression of GABA_A receptors subunits in immune cells. Therefore, we aimed to investigate the changes in GABA_A receptor subunit gene expression during macrophage differentiation and propofol administration in order to clarify the relationship between the expression of GABA_A receptors and the immunomodulatory effect of propofol.

Methods: Human acute monocytic leukemia (THP-1) cells were differentiated into macrophage-like cells (M0 THP-1); subsequently, M0 THP-1 cells were differentiated into inflammatory M1 macrophage-like cells (M1 THP-1). Propofol was administered during the differentiation into M1 THP-1 cells. Using reverse transcriptase polymerase chain reaction, we examined which GABA_A receptor subunit genes were expressed and whether there were changes in the gene expression during macrophage differentiation and propofol administration in THP-1 cells.

Results: The expression of the $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, and $\gamma 2$ subunits increased during differentiation into M0 THP-1 cells. The expression of the $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 2$, and δ subunits decreased and that of the $\gamma 1$ subunit increased during differentiation into M1 THP-1 cells. The gene expression of the $\alpha 1$, $\alpha 4$, and $\beta 2$ subunits increased upon administering propofol during differentiation into M1 THP-1 cells.

Conclusions: The gene expression of GABA_A receptor subunits changed during macrophage differentiation in THP-1 cells. The expressions of $\alpha 1$ and $\alpha 4$ increased following propofol administration during the differentiation into M1 THP-1 cells, which may indicate that the GABA_A receptor is involved in the immunosuppressive effects of propofol. This study can help in the choice of anesthetic agents for proinflammatory conditions such as highly-invasive surgery.

Background

Gamma-aminobutyric acid type A (GABA_A) receptors are ligand-gated anion channels that are activated when gamma-aminobutyric acid (GABA) binds to them. GABA is a major inhibitory neurotransmitter. The function of GABA has been well studied in the central nervous system; however, few studies are available on GABA expression related to physiological functions in other tissues. In particular, the expression of GABA_A receptors and their functions in immune cells have not been fully investigated. GABA_A receptor expression has been confirmed in monocytes [1], human acute monocytic leukemia cell lines (THP-1 cells), macrophages [2], and T-cells [3, 4]. Monocytes and macrophages produce GABA, which inhibits the production of inflammatory cytokines [5–7]. Hence, it has been hypothesized that the GABAergic signaling system is also present among immune cells, regulating cellular functions, such as cell proliferation, cytokine production, phagocytosis, and chemotaxis [2, 5, 7].

The GABA_A receptor is a pentamer consisting of three different subunits. There are 19 different types of GABA_A receptor subunits (α 1-6, β 1-3, γ 1-3, δ , ϵ , π , θ , and ρ 1-3). GABA_A receptors are mainly composed of 2 α , 2 β , and 1 γ or 1 δ [8]. Since the difference in composition determines the specific function and pharmacological properties of the channel, the identification of subunit expression in cells is crucial. The hypnotic effect of anesthetics is produced by the GABA_A receptors [9]. Propofol, a predominant hypnotic agent in the anesthetic field, is known to act on immune cells, resulting in immunomodulatory effects.[10, 11].

Macrophages are immune cells that are present in various tissues throughout the body. Monocytes differentiate into macrophages when they migrate to tissues throughout the body. During inflammatory processes, macrophages differentiate into M1 macrophages, which act as inflammatory cells, and M2 macrophages, which are responsible for tissue repair. These macrophages play an important role in the immune response [12, 13]. THP-1, a human acute monocytic leukemia cell line, has been widely used to investigate the function of human macrophages. THP-1 cells can differentiate into macrophage-like cells and M1/M2 macrophage-like cells [14, 15]. In a previous study using THP-1-derived macrophages, we found that propofol suppresses interleukin (IL)-6 and IL-1 β production without affecting M1/M2 differentiation and that GABA_A receptors could be involved in the suppression of cytokine production [16].

The expression of GABA_A receptor subunits on T cells and monocytes is altered by influenza infection. The administration of diazepam affects immune function and increases susceptibility to infection [17]. This finding indicates that changes in GABA_A receptor subunit expression can be involved in immune function and can exacerbate the immunosuppressive effect of diazepam.

Since the GABA_A subunits that are expressed in immune cells during inflammation have not been fully investigated, we aimed to analyze the expression of GABA_A receptor subunit genes in THP-1 cells during macrophage differentiation. Furthermore, we aimed to investigate the changes induced by propofol administration in order to clarify the precise mechanism of the immunomodulatory effect of propofol.

Methods

Aim

We aimed to analyze the expression of GABA_A receptor subunit genes in THP-1 cells during macrophage differentiation *in vitro*. Furthermore, we aimed to investigate the changes induced by propofol administration in order to clarify the precise mechanism of the immunomodulatory effect of propofol.

Materials

Roswell Park Memorial Institute (RPMI) 1640 medium, dimethyl sulfoxide (DMSO), lipopolysaccharide (LPS) from *Escherichia coli* strain O111:B4, and phorbol-12-myristate-13-acetate (PMA) were obtained

from Sigma-Aldrich (St. Louis, MO, USA). Interferon (IFN)- γ was obtained from R&D Systems (Minneapolis, MN, USA). Propofol was obtained from Wako Pure Chemical Industries (Osaka, Japan).

Cell culture and differentiation

THP-1 cells (ATCC; Manassas, VA, USA) resemble primary monocytes and macrophages in morphology and differentiation properties. When exposed to PMA, THP-1 cells adhere to culture plates and start differentiating into a macrophage-like phenotype; these cells are generally used to study human macrophage functions [14]. THP-1 cells were differentiated into macrophage-like cells (M0 THP-1) through incubation for 3 days with 200 nM PMA in RPMI 1640 supplemented with 5% fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 μ g/ml) [14]. M0 THP-1 cells were polarized into M1 macrophage-like cells (M1 THP-1) via incubation with 100 ng/ml of LPS and 20 ng/ml of IFN- γ [15].

To evaluate the effects of propofol on GABA_A subunit gene expression during M1 differentiation, M0 THP-1 cells were differentiated into M1 THP-1 cells in the presence of propofol (25–100 μ M) or in that of the solvent alone (0.05% DMSO). Under these experimental conditions, propofol had little effect on the viability of polarized THP-1 cells (> 95% by trypan blue staining).

Quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) assays

Total cellular ribonucleic acid (RNA) was extracted using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. Subsequently, complementary deoxyribonucleic acid (cDNA) was synthesized from the total RNA preparations using a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA) by following the guidelines. Further, qRT-PCR was performed using PowerUp SYBR Green Master Mix (Applied Biosystems) and specific primers (Takara; Table 1) on a 7500 Fast Real-Time PCR System (Applied Biosystems, CA, USA). Target messenger (m)RNA levels were normalized against housekeeping β -actin mRNA levels, and the expression level relative to that of the control was calculated using the $\Delta\Delta$ Ct method. The relative mRNA expression was expressed as fold expression over the control gene expression. The expression level with the control treatment was assumed to be 1.

Table 1

Primer sequences for quantitative real-time reverse transcription polymerase chain reaction

Gene	Forward primer (5'→3')	Reverse primer (5'→3')
β -actin	TGGCACCCAGCACAATGAA	CTAAGTCATAGTCCGCCTAGAAGCA
α 1	CAGCAAGTATGAACCACATGGAAC	ATGTGTGGAATGACTTGAACAGAGA
α 2	TCCCAAGTTTCATTCTGGCTTAACA	ACCAGTCCATGGCAGTTGCATA
α 3	GTCACAAGTGTCGTTCTGGCTCA	AGTCCATGGCCGTCGCATA
α 4	GTGCAGCATGTTGGCTTGTC	TCGCATAGATACACCTTTGCATGA
α 5	ATCTTGGATGGGCTCTTGGATG	CCGAAGCTGGTGACGTAGATG
α 6	AGGAGTCCGTCCCAGCAAGA	GTTGACAGCTGCGAACTCGATAAG
β 1	TCTGCAGCCAGAGTCGCACTA	ATACTCCAGCAGAGCCAGGAACA
β 2	CTTTGAGTTCCCAAACCAAATGTC	TGGAAGTGTCAACTTGCTTCAAATG
β 3	GCAGAAGTGCCTCTGGAAATTGA	TCCACTCCGGTAACAGCCTTG
γ 1	GCAGCCTTGATGGAATATGGAAC	TGGATCCAGGATGGAGACCAG
γ 2	TCTGGCAAATCTCTGTGCTG	TCACTTGACAACACCTATGTGAGAA
γ 3	TGGATCACCACACCCAATCAG	ATCAGCGGGCAGGAGTGTTTC
δ	GTGCATGCTGGACCTGGAGA	CGGTAGCTGGTGATGGTGAAC

Statistical analysis

Values are expressed as the mean \pm Standard Deviation (SD), and results were obtained from six separate experiments. Differences between two groups were analyzed by an unpaired two-tailed t-test, whereas differences among multiple groups were analyzed by a one-way analysis of variance (ANOVA), followed by Bonferroni's *post hoc* test. All the statistical analyses were performed using GraphPad Prism software program V. 6.00 (GraphPad Software; La Jolla, CA, USA), with $P < 0.05$ defined as statistically significant.

Results

Increase in the gene expression of the α 1, α 4, β 1, β 2, γ 1, and γ 2 GABA_A receptor subunits during the differentiation into M0 THP-1 cells

GABA_A receptor subunit gene expression was investigated during macrophage differentiation. The gene expression of the α 1, α 4, β 1, β 2, γ 1, and γ 2 GABA_A receptor subunits in THP-1 cells significantly increased

during the differentiation into M0 THP-1 cells. The expression of $\alpha 3$, $\alpha 5$, $\alpha 6$, $\beta 3$, and $\gamma 3$ was not detected in THP-1 cells. The expression of $\alpha 2$ and δ did not change during macrophage differentiation (Fig. 1).

Decrease in the expression of the $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 2$, and δ subunits and increase in the expression of the $\gamma 1$ subunit during the differentiation into M1 THP-1 cells

We examined GABA_A receptor subunit gene expression in THP-1 cells during M1 differentiation. The gene expression of $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 2$, and δ significantly decreased during the differentiation into M1 THP-1 cells, while that of $\gamma 1$ significantly increased (Fig. 2).

Increase in the expression of the $\alpha 1$, $\alpha 4$, and $\beta 2$ GABA_A receptor subunits following the administration of propofol during the differentiation into M1 THP-1 cells

We evaluated the effect of propofol on the expression of GABA_A receptor subunits during M1 differentiation in THP-1 cells. Propofol significantly increased the $\alpha 1$, $\alpha 4$, and $\beta 2$ gene expression, while that of $\beta 1$, $\gamma 1$, $\gamma 2$, and δ was not affected by propofol administration (Fig. 3).

Discussion

In this study, the gene expression of the $\alpha 1$, $\alpha 2$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, and δ GABA_A receptor subunits was detected in THP-1 cells. The gene expression of $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, and $\gamma 2$ increased during macrophage differentiation. The gene expression of $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 2$, and δ decreased during M1 differentiation; additionally, propofol administration increased the expression of $\alpha 1$, $\alpha 4$, and $\beta 2$ during M1 differentiation.

GABA is a major inhibitory neurotransmitter in the mammalian central nervous system. GABA_A receptors, the primary target of GABA, are pentameric complexes consisting of three different subunits. Various combinations of GABA_A receptor subunits determine receptor function. Mammals express 20-30 different GABA_A receptor isoforms. The most common combination of GABA_A receptor subunits consists of two α subunits, two β subunits, and one γ or δ subunit [18]. In this study, we investigated 13 GABA_A receptor subunits: $\alpha 1-6$, $\beta 1-3$, $\gamma 1-3$, and δ . Most of the hypnotic effects of anesthetic agents are produced by the activation of GABA_A receptors. Classical benzodiazepines, such as diazepam, bind to two distinct binding sites on the receptor. Benzodiazepines bind to the α - γ subunit interface present in the extracellular domain of the receptor. Benzodiazepines also bind to the β - α interface and γ - β interface, which are present in the transmembrane domain. Propofol binds to the β - α interface present in the transmembrane domain of the receptor [19].

GABA_A receptors are present on immune cells, and GABA_A signaling modifies immune function. GABA_A receptors have been found on cluster of differentiation (CD)4+ and CD8+ T cells, macrophages, monocytes, and THP-1 cells [1–4]. The administration of diazepam in a mouse pneumonia model leads to immunosuppression, resulting in higher mortality, while the administration of the GABA_A receptor antagonist, bicuculline, counteracts the immunosuppressive effect of diazepam and decreases mortality [20]. Another study reported that benzodiazepines are involved in immunosuppressive effect, resulting in increased mortality and the incidence of pneumonia [21]. The expression of GABA_A receptor subunits on T cells and monocytes is modified by influenza infection, and diazepam administration affects immune function and increases susceptibility to infection [17]. These results indicate that the expression of GABA_A receptor subunits can be modified by external stimuli, such as inflammation, differentiation, and drug administration, including the administration of intravenous anesthetics.

Our previous study showed that via the effects on GABA_A receptors in THP-1 cells, propofol suppresses the production of inflammatory cytokines, IL-6 and IL-1 β , during the differentiation into inflammatory M1 macrophage-like cells without affecting M1 differentiation [16]. The present study show that the gene expression of α 1, α 4, β 2, and other subunits of GABA_A receptors decreased during M1 differentiation, whereas addition of propofol increased the gene expression of α 1, α 4, and β 2 subunits during M1 differentiation. Taken together, our previous and present data suggest that the suppression of inflammatory cytokines production during M1 differentiation in THP-1 cells by propofol administration may be associated with increasing the gene expression of α 1, α 4, and β 2 subunits.

GABA_A receptors have already been reported to be involved in inflammatory diseases, such as asthma, intestinal inflammation, and pulmonary fibrosis. The GABA_A receptor, α 1, is observed in human alveolar macrophages and monocytes and is responsible for diazepam-induced immunosuppression [17, 20]. When the GABA_A receptor, α 4, is knocked out in mice suffering from asthma, lung inflammation and airway reactivity deteriorate further [22]. In mice with stress-induced intestinal inflammation, α 1, α 4, and α 5 GABA_A receptor agonists exert anti-inflammatory effects, while α 3 receptor agonists exacerbate inflammation [23]. Diazepam administration activates GABA_A α 4 receptors, thereby suppressing LPS-induced lung injury and the development of pulmonary fibrosis [24]. These results suggest that α 1 and α 4 GABA_A receptors are involved in the suppression of inflammatory responses. In addition to such previous data, our data suggest that propofol suppresses production of inflammatory cytokines in THP-1 cells during the M1 differentiation and propofol increases gene expressions of α 1, α 4, and β 2 subunits of GABA_A receptors during M1 differentiation. Taken together, it is quite possible that propofol exerts its immunosuppressive effect by increasing expressions of α 1 and α 4 subunits of GABA_A receptors.

This study has several limitations. First, an artificial THP-1 cell line was used; therefore, macrophages from human peripheral blood should be examined in a clinical setting in the next study, as the results from peripheral blood can be close to those acquired in actual situations and may easily be applied in clinical therapy. Second, only gene expression was analyzed in this study; therefore, changes should be examined at the protein level to clarify the precise mechanism of immunosuppressive effect via the

GABA_A receptor. Third, we analyzed only 13 major subunits; however, all 19 GABA_A receptor subunits should be investigated in order to acquire more accurate results. Further studies are required to address these limitations.

Conclusions

The gene expression of GABA_A receptor subunits was modified during macrophage differentiation in THP-1 cells. In particular, the gene expression of $\alpha 1$ and $\alpha 4$ was increased by propofol administration during M1 differentiation. These results suggest that the immunomodulatory effect of propofol may be related to changes in the GABA_A receptor subunit gene expression. These results can help clinicians choose appropriate anesthetic agents for proinflammatory conditions such as highly-invasive surgery.

Abbreviations

GABA = gamma-aminobutyric acid type

GABA_A = gamma-aminobutyric acid type A

THP-1 cells = human acute monocytic leukemia cells

RPMI = Roswell Park Memorial Institute

DMSO = dimethyl sulfoxide

LPS = lipopolysaccharide

PMA = phorbol-12-myristate-13-acetate

IFN = interferon

FBS = fetal bovine serum

qRT-PCR = quantitative real-time reverse transcription polymerase chain reaction

RNA = ribonucleic acid

cDNA = complementary deoxyribonucleic acid

SD = standard deviation

ANOVA = one-way analysis of variance

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data used to support the findings of this study are available from the corresponding author upon request. Our dataset of 13 genes does not qualify as high-throughput data, and thus, is not suitable for submission to the Gene Expression Omnibus database.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

All authors contributed to the design of the experimental protocols. Material preparation, data collection, and analysis were performed by TK, AY, and MF. The first draft of the manuscript was written and edited by TK and IK, and all the authors commented on the previous versions of the manuscript. All the authors have read and approved the final version of the manuscript.

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Figures

Fig. 1

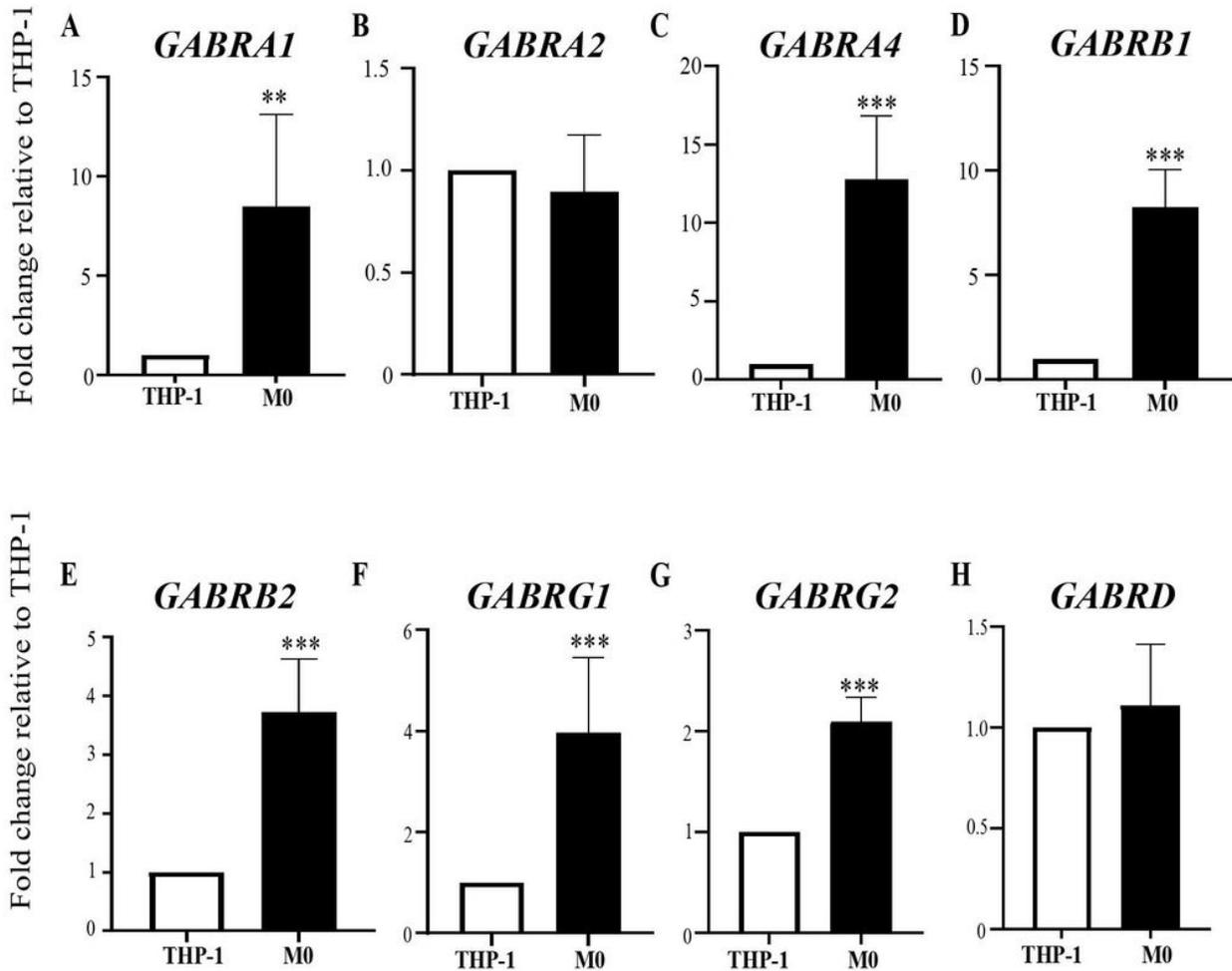


Figure 1

Changes in GABAA receptor subunit gene expression during differentiation of THP-1 cells into M0-THP-1 cells. THP-1 cells were differentiated into M0-THP-1 cells. (A-H) RT-PCR assays of GABAA subunit mRNA levels in THP-1 cells. The gene expression of the $\alpha 1$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, and $\gamma 2$ GABAA receptor subunits was increased during differentiation into M0 THP-1 cells. Data were normalized relative to β -actin mRNA (internal control) and presented as mean \pm SD (n = 6 per group). **P < 0.01, ***P < 0.001 compared with control cells by an unpaired t-test. Abbreviations: GABAA, gamma-aminobutyric acid type A; THP-1, human acute monocytic leukemia cell line; M0-THP-1, macrophage-like cells; RT-PCR, real-time reverse transcription polymerase chain reaction; mRNA, messenger ribonucleic acid; SD, standard deviation

Fig. 2

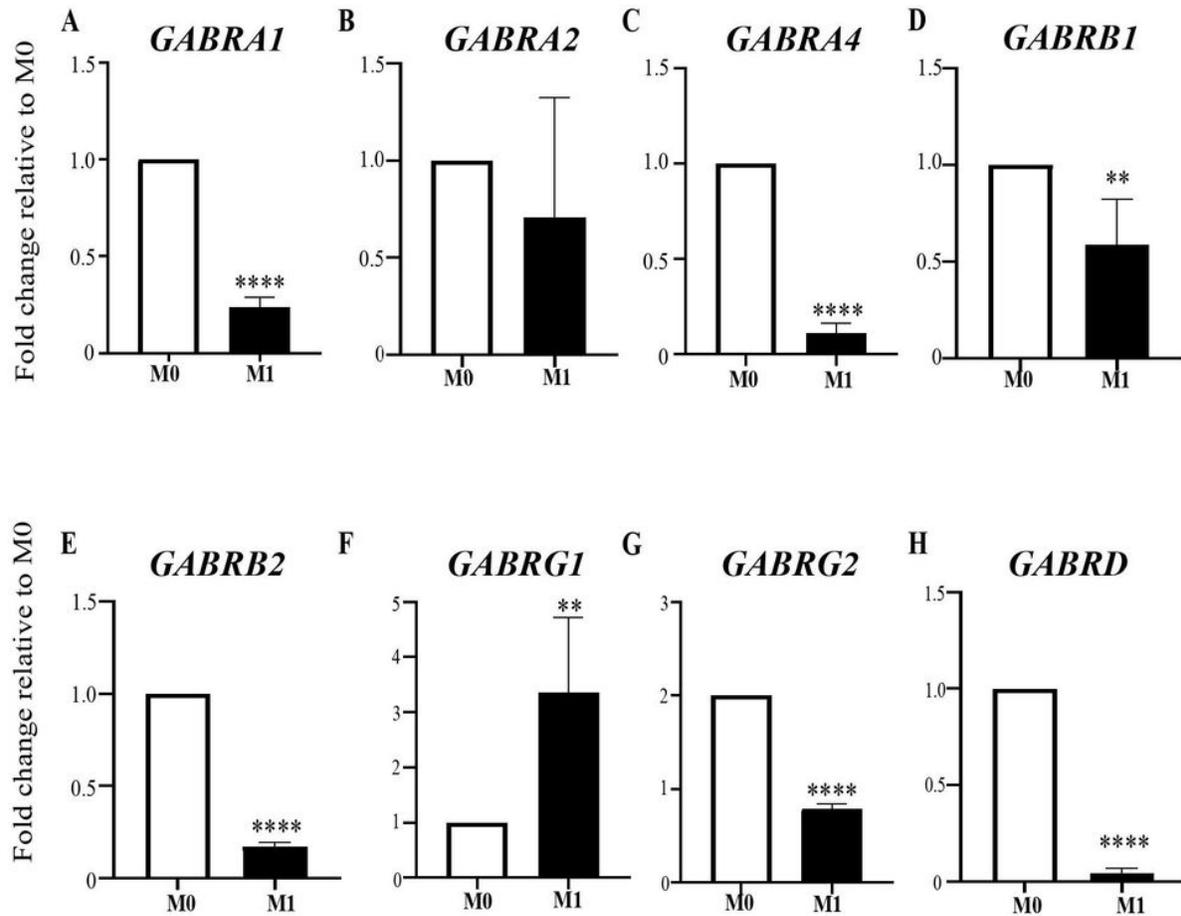


Figure 2

Changes in GABA_A receptor subunit gene expression during differentiation of M0-THP-1 cells into M1-THP-1 cells. M0-THP-1 cells were differentiated into M1-THP-1 cells. (A-H) RT-PCR assays of GABA_A subunit mRNA levels in THP-1 cells. The expression of the α 1, α 4, β 1, β 2, γ 1, and γ 2 GABA_A receptor subunits was decreased during differentiation into M0 THP-1 cells. Data were normalized relative to β -actin mRNA (internal control) and presented as mean \pm SD (n = 6 per group). **P < 0.01, ****P < 0.0001 compared with control cells by an unpaired t-test. Abbreviations: GABA_A, gamma-aminobutyric acid type A; THP-1, human acute monocytic leukemia cell line; M0-THP-1, macrophage-like cells; M1-THP-1, M1 macrophage-like cells; RT-PCR, real-time reverse transcription polymerase chain reaction; mRNA, messenger ribonucleic acid; SD, standard deviation

Fig. 3

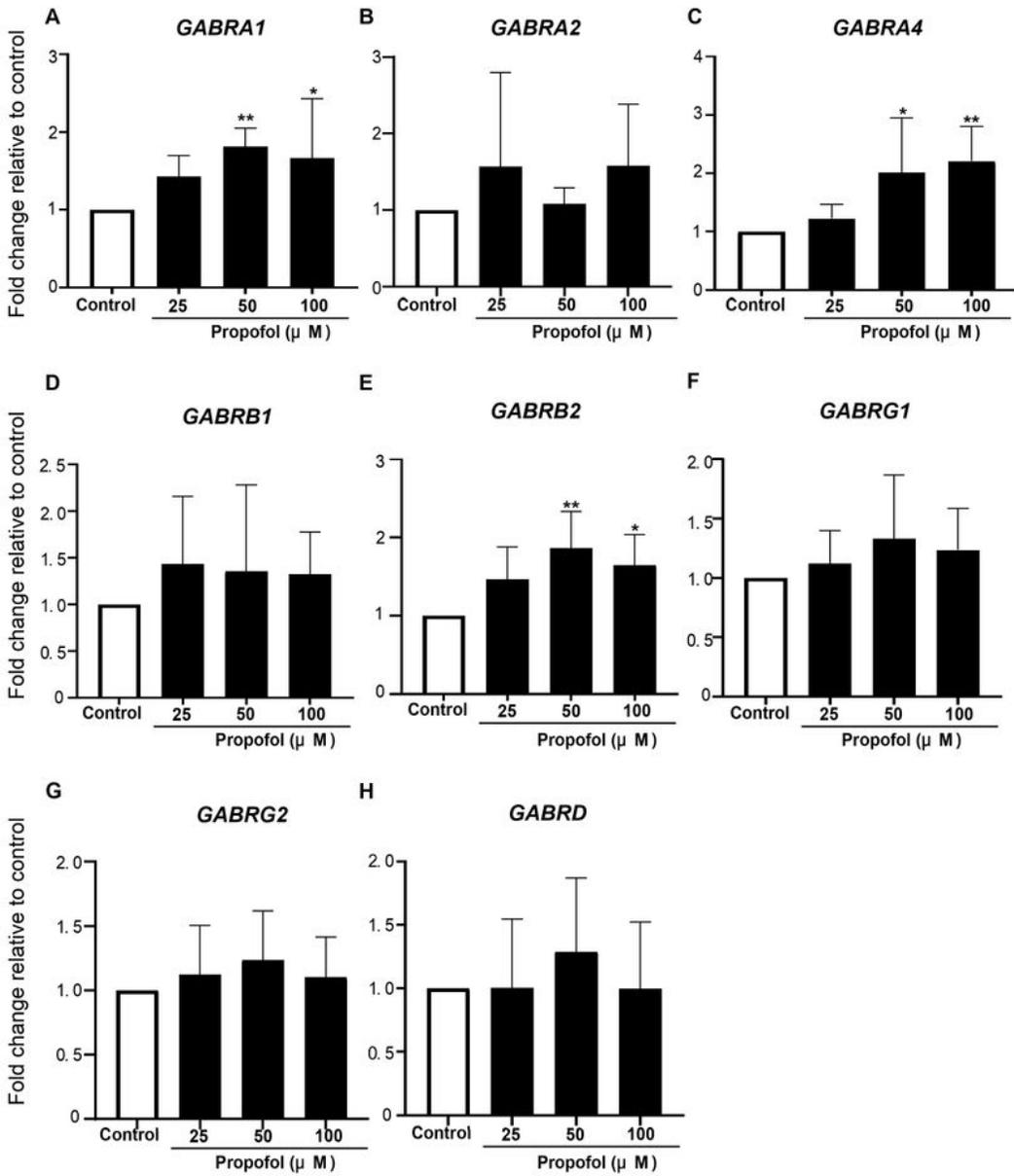


Figure 3

Changes in GABA_A receptor subunit gene expression following the administration of propofol during M1 differentiation. M0-THP-1 cells were differentiated into M1-THP-1 cells in the presence of 0.05% DMSO as the control solvent or in the presence of propofol (25-100 μ M). (A-H) RT-PCR assays of GABA_A subunit mRNA levels in THP-1 cells. The administration of propofol (50, 100 μ M) during differentiation into M1 THP-1 cells increased the gene expression of the α 1, α 4, and β 2 GABA_A receptor subunits. Data were

normalized relative to β -actin mRNA (internal control) and presented as mean \pm SD (n = 6 per group). *P < 0.05, **P < 0.01, compared with control cells by a one-way ANOVA and Bonferroni's post hoc test. Abbreviations: GABAA, gamma-aminobutyric acid type A; THP-1, human acute monocytic leukemia cell line; M0-THP-1, macrophage-like cells; M1-THP-1, M1 macrophage-like cells; DMSO, dimethyl sulfoxide; RT-PCR, real-time reverse transcription polymerase chain reaction; mRNA, messenger ribonucleic acid; SD, standard deviation; ANOVA, analysis of variance