PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2021,48(4):465~474
www.pibb.ac.cn

Study on Metformin Regulating The Balance of Excitatory and Inhibitory of Primary Visual Cortex and Improving Visual Function*

CAI Yang, LI Bao-Wei, ZHOU Yi-Feng**
(*School of Life Sciences, University of Science and Technology of China, Hefei* 230027, China)

Abstract The dynamic balance of excitation and inhibition systems in the cerebral cortex determine the response characteristics of neurons to the stimulation. It has been reported that metformin can induce the postsynaptic clustering of gamma-aminobutyric acid (GABA) receptors and enhance inhibition in the nervous system. Here we explore the regulatory effect of metformin on the balance of the excitatory and the inhibitory system of the primary visual cortex, and its potential to improve visual function in mice. Adult male mice were treated with metformin (metformin group) and normal saline (control group) for 3 weeks of intragastril administration. We found that metformin can significantly increase the production of vesicle GABA transporter (VGAT) and postsynaptic inhibitory receptor-related protein (Gephyrin). Furthermore, it significantly reduced the expression of postsynaptic excitatory receptors GluA1 and GluN1. The data also demonstrated that the multichannel electrode recording shows that Baseline Response and Maximum Response of the primary visual cortex were significantly decreased, while under the treatment of metformin the signal-to-noise ratio, directional and orientation bias were significantly increased. Our finding reveals that metformin could reduce the excitation synapse, enhance the inhibition synapse, and adjust the balance of excitation-inhibition of the primary visual cortex, thus improving information processing ability and enhancing visual function.

Key words metformin, primary visual cortex, multi-channel electrode, excitation system, inhibition system
DOI: 10.16476/j.pibb.2020.0289

Metformin (N, N-dimethylbiguanide) is a widely used clinical drug with numerous benefits, especially for diabetes[1]. In addition to its antidiabetic potential, metformin has been proved that it can cross the blood-brain barrier with specific effects on the central nervous system (CNS)[2-3]. Metformin, a potent AMPK (AMP-activated protein kinase) activator, has been found to play a neuroprotective role in cerebral cortical cells by inhibiting apoptosis[4]. Furthermore, it has been reported that metformin can enhance the formation of spatial memory by promoting neurogenesis[5-6]. While, some studies presented that the long-term treatment of metformin can improve the healthy life span and life span of the mice with Huntington disease[7-8]. Thus metformin has become an effective drug candidate for the treatment of many central nervous system diseases. However, the mechanisms of metformin on central nervous system are mostly unknown. Recently, it was proposed that metformin can enhance the ability of postsynaptic neurons to receive transmitters *via* accelerating or promoting the transport of intracellular GABAA receptors to the plasma membrane[9]. Thus the literature suggested that metformin may protect the nervous system by affecting the neurotransmitter system. Considering these facts, the primary objective of the present study was to evaluate the adjustment ability of metformin with respect to the balance of

* This work was supported by grants from The National Natural Science Foundation of China (31571074, 91749102).
** Corresponding author.
Tel: 86-13033082368, E-mail: zhouy@ustc.edu.cn
Received: August 7, 2020 Accepted: November 2, 2020

excitatory and inhibitory of primary visual cortex and improving visual function.

Primary visual cortex exists in all mammalian cortex, and currently, is one of the most studied and clearest cortex. In the primary visual cortex, inhibition is considered to provide a balance against excitement, which maintains the nervous system in a certain dynamic equilibrium state[10-11]. Extracellular recording of visual cortex show that the "diversity" combination of inhibition and excitation affects the peak response of neurons to different stimuli[12], and the information processing process in the visual cortex[13-14]. Besides this, the decline of inhibition system or enhancement of excitation systems will destroy the balance between excitation and inhibition, which will lead to the increment of the neuron fire level, decrement of signal-to-noise ratio, and weaken information processing ability[11, 15-16]. Hence the drug with benefits of having neuroprotective and/or regulating effect will be a major breakthrough in the visual improvement.

This study is based on the maximum recommended dose of metformin for a human (2 000 mg/d) which is equivalent to 20 mg/kg/d. While the commonly used dose of metformin in mice is about 250 mg/kg/d[17-19]. To determine the effect of metformin on proteins related to the excitation and inhibition system of the primary visual cortex, Western blotting was carried out. We mainly analyzed the proteins related to inhibitory synapses, vesicle GABA transporter (VGAT) and postsynaptic inhibitory receptor-related protein (Gephyrin); and the proteins related to excitatory synapses, excitatory receptors (GluA1, GluN1), postsynaptic dense protein (PSD95) and neurotransmitter release related protein (HSP90). In addition, the neuron fire was assessed by determining the baseline response and maximum response levels, the information processing ability was evaluated by signal-to-noise ratio(SNR), while the neuron tuning characteristics was measured by determining directional and orientation bias (DB, OB).

1 Materials and methods

1.1 Animal

16 C57BL6 mice were used in this experiment (control group n=6; metformin group n=10), age of 7 weeks. Mice were treated with metformin (250 mg/kg, D150959 Sigma-Aldrich, dissolved with 0.3 ml normal saline) by repeated intragastric administration for 3 weeks (control group: 0.3 ml normal saline; metformin group: 250 mg/kg/d metformin).

1.2 Electrophysiological recording

The experimental stimulus was written by Matlab, which was assisted by the psychophysics extension toolkit and video toolkit. The experimental stimuli were displayed on a CRT monitor by with garmma value corrected nonlinear function (1 024× 768 pixel, 100 Hz, G220, Sony, Japan). During the experiment, the average brightness of the computer screen was set as 26 cd/m^2 and contrast was set at 100%. The screen was 33 cm away from the eyeball. Initially, the position and size of the receptive field of the target cells were determined. This step was realized by the control system, which displayed a series of experimental stimuli on the monitor. These experimental stimuli represent the cell response with the help of moving sinusoidal grating as a stimulus to detect direction and orientation tuning ability. It contains 13 directional stimuli (0, 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360) and blank control, 10 try cycles, and one blank stimulus were presented randomly in each cycle. There was a time interval of 5 s between different stimuli, to record the self-fire level of neurons and prevent the cell from adapting. The extracellular electrical signal was firstly filtered by an amplifier (DAGAN, Mineapolis, MN, USA) and then amplified 10 000 times. After that, the amplified signal passes through a window discriminator (Winston Electronics, StLouis, M0, USA) and finally, stored in the computer for offline analysis after digital-to-analog conversion by data acquisition card (National Instrument, Austin, TX, USA).

1.3 Data analyses

Data fitting was completed by MATLAB's toolkit. Statistical analysis was carried out using GraphPad Prism 8 software. We mainly analyzed two parameters (1) direction and orientation bias (DB, OB) and (2) baseline response and maximum response of neurons. By comparing the changes in OB, OD, baseline response and maximum response, we can examine the effect of metformin on neuron characteristics. The value of OB and DB represents the strength of neurons tuning ability *i. e.* when OB and DB are minimized (0), it shows that neurons have

no tuning ability while with the maximum value (1), the neurons have tuning ability only in one direction or orientation. Similarly, the values of baseline response and maximum response represent the strength of neuronal activity, such as larger the value, stronger the response. In order to study the information processing ability of neurons, we also analyzed the signal-to-noise ratio (the quotient of the magnitude of the cell's response to the optimal stimulus (maximum response) divided by the magnitude of the cell's spontaneous response (baseline response)). In the analysis of signal-to-noise ratio, all average baseline response below 1 spike is set to 1 spike.

1.4 Western blot

After the electrophysiological experiment, the brain was taken quickly, and the primary visual cortex was separated and frozen at −80℃. After completing the electrophysiological experiments, samples (control group n=4; metformin group n=4) were treated in homogenizers respectively. The samples were placed in ice bath with buffer containing PMSF for 30 min, and homogenates were centrifuged at 13 000 r/min for 10 min. The BCA protein quantitative kit was used to measure protein concentration. Subsequently, performing standard SDS-PAGE and Western blotting. The following primary antibodies were used. CST: mAb#5704-GluN1, mAb#13185-GluA1; Abcam: ab2723-PSD95, ab26113-GAD65; Proteintech: 13171-1-A-HSP90; SynapticSystems-Cat#: 131003-VGAT, 147021-Gephyrin. The secondary antibody was used (1∶1 000). Membranes were developed with enhanced chemiluminescence (ECL) solution (GE Healthcare, Chicago, IL, USA) and the ChemiDoc imaging system (Bio-Rad). Finally, Adobe Photoshop was used to quantify the samples.

2 Results

2.1 Metformin decreases the expression of GluA1 and GluN1, while has no effect on PSD95 and HSP90 in excitatory neurotransmitter system

Firstly, we examined the effect of metformin on the excitatory receptor and its receptor-associated proteins of control group (n=4) and metformin group (n=4). We mainly focus on two main Glu receptors GluN1 and GluA1, neurotransmitter release related protein HSP90 and postsynaptic dense protein PSD95. Our results proposed that metformin significantly decreased the expression levels of GluA1 and GluN1 compared to control group as shown in Figure 1a and 1b (Multiple t-test, GluA1: P<0.0089, GluN1: P< 0.0066). Also, it has no significant effect on the formation of PSD95 and HSP90 compared to control group as shown in Figure 1c and 1d (Multiple t-test, PSD95: P>0.36, HSP90: P>0.21).

2.2 Metformin increases the expression of Gephyrin and VGAT in inhibitory neurotransmitter system

Secondly, we further analyzed the effect of metformin on vesicle GABA transporter VGAT and postsynaptic inhibitory receptor-related proteins Gephyrin. The presented results illustrate the significantly increasing effect of metformin on the expression level of Gephyrin and VGAT as compared to control shown in Figure 2a and 2b (Multiple t-test, Gephyrin P< 0.003, VGAT P< 0.007).

2.3 Metformin enhances the signal−to−noise ratio *via* decrease the baseline response and the maximum response of neurons

In order to further explore the impact of metformin on the visual system, we recorded the changes in the response intensity of neurons to grating stimulation through electrophysiological techniques (Figure 3a, b). We further evaluated the baseline response and maximum response of neurons in the control and metformin groups. 16 mice were used in the experiment, 98 cells were recorded in the control group (n=6); and 72 cells were recorded in the metformin group (n=10). The results showed that metformin significantly decreased the baseline response and maximum response of neurons (Figure 3b left, middle, Mann-Whitney test, baseline response: P< 0.024; maximum response: P< 0.045). The changes of response intensity of neurons to grating stimulation, compared with the control group, the response intensity of baseline response and maximum response of neurons induced by metformin decreased significantly (Figure 3a). In order to measure the influence of baseline response and maximum response change on information processing ability, we further analyzed the changes of signal-to-noise ratio of neurons. The results showed that, under the action of metformin, the signal-to-noise ratio of primary visual cortex neurons increased significantly (Figure 3b right, SNR: P<0.0005).

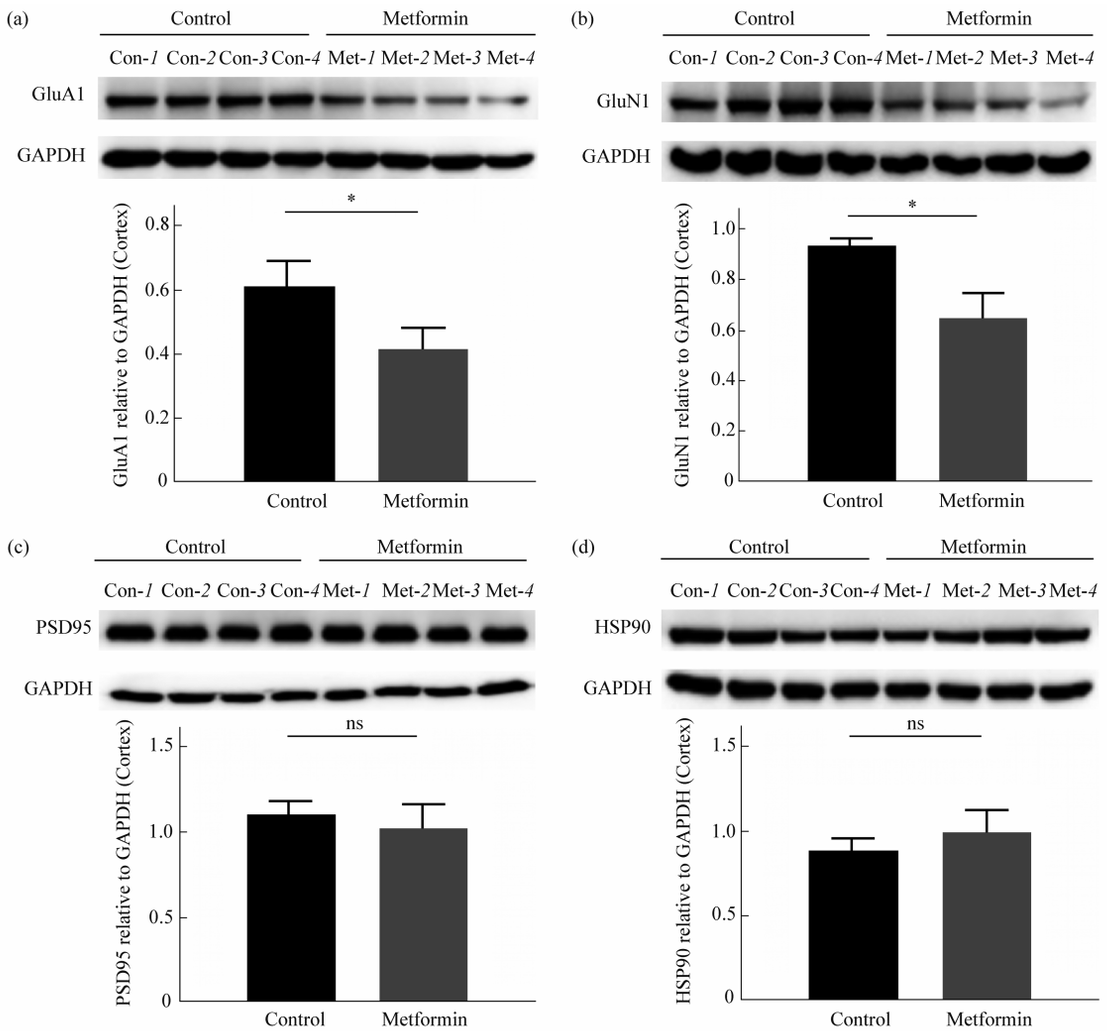

Fig. 1　Effect of metformin on excitatory synaptic related proteins

Effect of metformin on GluA1 (a), GluN1 (b), PSD95 (c) and HSP90 (d) in primary visual cortex. Multiple *t*-test having $P<0.05$ was performed for statistical analysis. The data are presented as ($\bar{x} \pm s$).

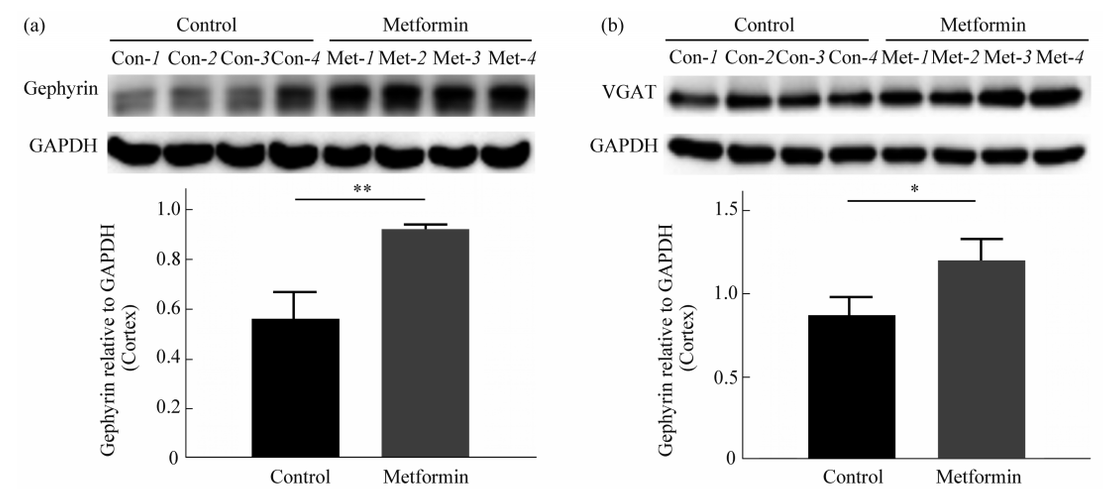

Fig. 2　Effect of metformin on inhibitory synaptic related proteins

Effect of metformin on Gephyrin (a) and VGAT (b) in primary visual cortex. Multiple *t*-test having $P<0.05$ was performed for statistical analysis. The data are presented as ($\bar{x} \pm s$).

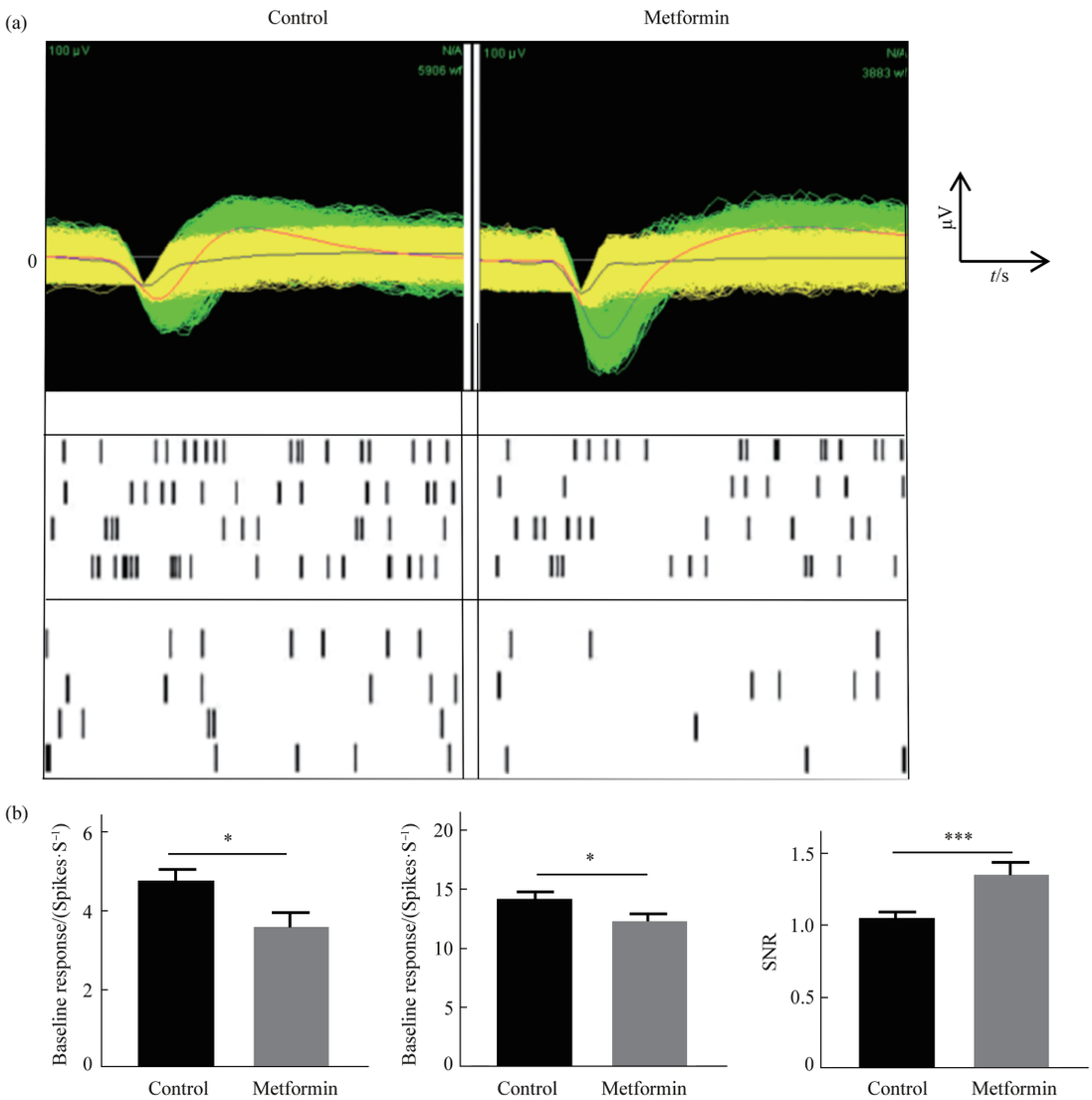

Fig. 3　Changes of nerve fire intensity and signal−to−noise ratio

(a) In a recording cycle, waveforms traces of primary visual cortex neurons stimulated by grating stimulation. Top: yellow for baseline response, green for maximum response. (Control group: baseline response=3 935, maximum response=1 971; Metformin group: baseline response=2 716, maximum response=1 167). Middle and lower, spike raster figure of neurons stimulated by four times, middle for maximum response, lower for baseline response. (b) The histogram of the baseline response (left), maximum response (middle), signal-to-noise (right) in the recorded neurons. The Mann-Whitney test having $P< 0.05$ was used for statistical analysis. The data are presented as ($\bar{x} \pm s$).

2.4　Metformin enhances the orientation tuning ability of neuron

Finally, we analyzed the effect of metformin on the visual function. We recorded the changes of neuron orientation tuning ability under the action of metformin. 16 mice were used in the experiment, 98 cells were recorded in the control group (n=6), and 72 cells were recorded in the metformin group (n=10). Compared with the control group, we analyzed the change of orientation bias and direction bias index. The results show that, DB and OB were significantly increased compared to the control group (Figure 4b, OB: $P< 0.0048$, DB: $P< 0.0001$). Figure 4a represents the two neurons with different tuning abilities in the control group and metformin group, and their tuning ability has been enhanced under the action of metformin.

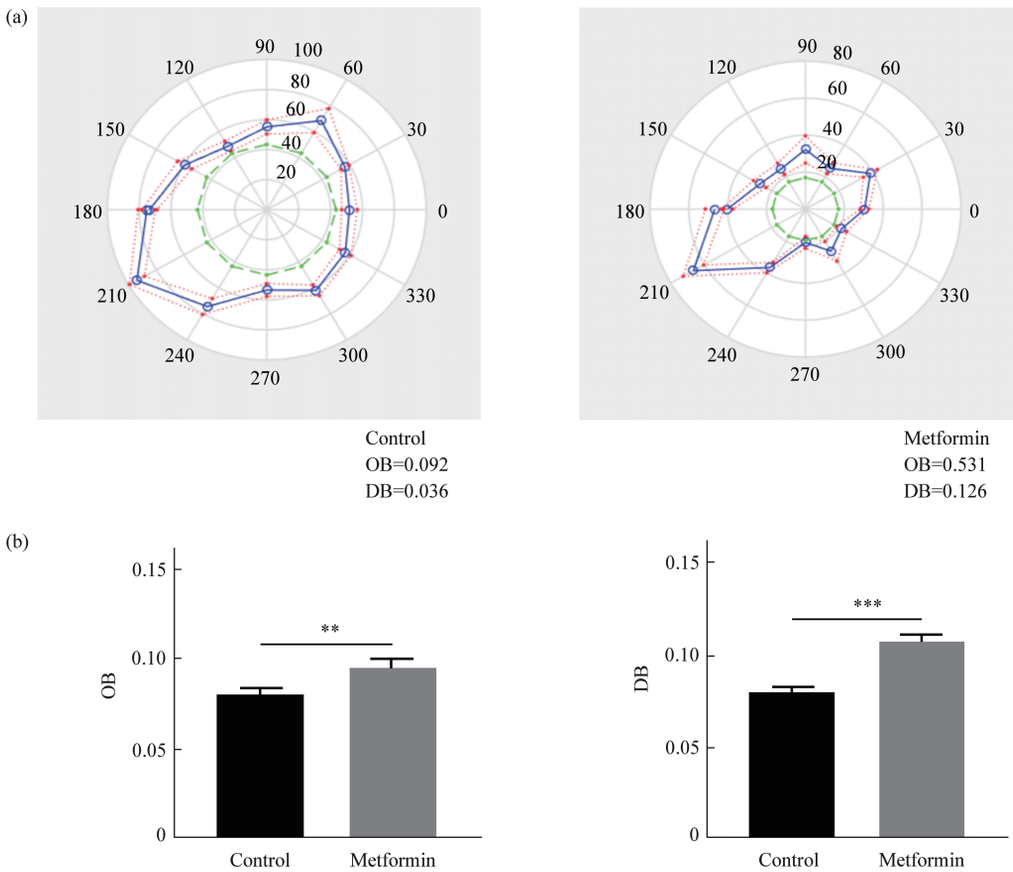

Fig. 4　Effect of metformin on orientation and direction bias

(a) Schematic diagram of orientation tuning of two typical neurons. (b) Statistical results of changes in direction and orientation bias.

3　Discussion

In this study, we found that metformin can significantly decrease the expression level of excitatory receptor GluA1 and GluN1. Beside this, it can also increase the expression of inhibitory receptor-related protein Gephyriny and vesicle GABA transporter protein VGAT. The electrophysiological recording shows that baseline response and maximum response of neurons in the primary visual cortex decreased significantly, while the signal-to-noise ratio, direction bias and orientation bias increased significantly. These results indicate that metformin can regulate the excitation and inhibition balance of the primary visual cortex and improve visual functions.

In the mammalian cerebral cortex, the dynamic balance of excitation and inhibition system determines the response characteristics of neurons to the stimulation. Extracellular electrophysiology reveals that the combination of inhibition and excitement not only affects the characteristics of neurons themselves but also affects the information processing ability in the visual cortex[12, 14-15, 20-21]. The interaction between excitation and inhibition is one of the key factors that determine the information processing ability of cortical neurons[12, 20, 22]. In the nervous system, inhibition is considered to provide a balance against excitement which maintains the nervous system in a certain dynamic equilibrium state.

Previous studies have shown that metformin can pass through the blood-brain-barrier and have specific effects on the central nervous system. Metformin exposure can play a neuroprotective role or improvement of spatial memory. Some tests in these studies are related to the visual system[23-24]. Therefore, it is significant to explore the effect of metformin on the visual cortex. In the proposed study, we examined the potential of metformin on the balance of excitatory and inhibitory of primary visual

cortex to better understand the metformin improving visual function.

The excitability of the nervous system is mainly related to the activation of excitatory receptors. Nervous systems use excitatory cell assemblies to encode and represent sensory percepts. However, excitatory abnormalities occur with over-activation of excitatory receptors[22, 25]. This not only increases the neuron fire level but also reduces the information processing ability of neurons and the visual cortical function[16, 26]. Here, we found that metformin reduces the expression of the Glu receptor, which is different from the change of GABAA receptor (metformin promote GABAA receptors aggregated to the postsynaptic membrane, but the total amount of receptors remains unchanged[9]. Furthermore, there was no significant change in the protein level of PSD95(related to the aggregation of excitatory receptors[27]) and HSP90(related to neurotransmitter release[28-31]) in the metformin group. These result indicate that compensatory clustering of Glu receptors did not occur in the postsynaptic membrane when the content of Glu receptors decreased, which reduces the ability of the postsynaptic neuron to receive excitatory neurotransmitter. Besides this, there was no obvious effect on the release of excitatory neurotransmitters. In general, metformin may weaken the excitability of the nervous system.

We further found that the contents of Gephyrin and VGAT increased significantly under the treatment of metformin. Gephyrin is a multifunctional protein that can self-assemble to form protein scaffolds in inhibitory synapses and take charge of GABA receptors aggregates in postsynaptic[32-35]. Previous experiments showed that when the RNAi removed, Gephyrin would strongly affect the clustering of GABAA receptor in the plasma membrane[36-38]. In our experiment, the expression levels of Gephyrin increased which indicates that metformin not only promotes the transport of intracellular GABAA receptors to the plasma membrane[9]. Moreover, the increase of Gephyrin content also provides a possibility to clustering of GABAA receptors. In addition, increment in VGAT content indicated that metformin enhanced the aggregation and release ability of GABA in the presynaptic membrane[39-40]. In summary, we believe that metformin can enhance the inhibitory synapses of the cortex in many ways.

To explore the effect of the change of cortical excitatory inhibition system on neuron response under metformin, we analyzed the changes of neuron fire and tuning characteristics by electrophysiological techniques. The results shows that baseline response and maximum response of neurons were significantly decreased under the treatment of metformin. This indicated that metformin may regulate the excitation-inhibition balance of the nervous system by strengthening the inhibitory (GABAergic) synapses, while weakening the excitatory (Glutamatergic) synapses, and thus weakening the neuronal fire. What is the effect of the change of neuron fire characteristics? Further analysis shows that the decrease of baseline response and maximum response of neurons leads to the increase of signal-to-noise ratio. As a parameter reflecting the information processing ability of the nervous system, the increase of signal-to-noise ratio indicates that metformin enhances the information processing ability of primary visual cortex[15, 41-43]. Finally, we have analyzed the effect of metformin on the function of neurons in the primary visual cortex, the results represent that orientation and direction bias of neurons were significantly enhanced by metformin, which indicated that metformin could improve the function of neurons in primary visual cortex.

4 Conclusion

In summary, we demonstrated that metformin can regulate the excitation-inhibition balance of the primary visual cortex by strengthening the inhibitory (GABAergic) system and weakening the excitatory (Glutamatergic) system, and improving information processing ability. This effect of metformin improves the functional characteristics of neurons in the primary visual cortex. However, due to the lack of experimental design and time constraints, there are still many shortcomings and deficiencies in this study. Firstly, in the aspect of the effect of metformin on the excitation-inhibition system, we only reflected the changes of synapse-related proteins in visual cortex after long-term treatment of metformin. From the results we found that metformin can change the expression of related proteins. In order to fully understand the role of metformin in synapses, we also need to explore the effect of metformin on gene level. In this way, the effect of metformin on the physiological function of this gene can be analyzed

more comprehensively. Secondly, we only used Western blot to test the changes of protein expression. In order to reduce the experimental errors and increase the reliability of the results, we should carry out various verifications through different experimental techniques, such as immunohistochemical experiments. Thirdly, as it is recognized that AMPK is the main target of metformin, so how to find the connection between synapse-related proteins and AMPK needs further study. In addition, there are some limitations in exploring the effects of metformin on neurons through electrophysiological records. We hope that we can accurately explore the effect of metformin on the response characteristics of different types of neurons by combining photogenetic methods. In general, this study has only achieved phased results. Still, it is necessary to combine a variety of experimental techniques to get more convincing and reliable research.

References

[1] Zhou G, Myers R, Li Y, *et al*. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest, 2001, **108**(8): 1167-1174

[2] Labuzek K, Liber S, Gabryel B, *et al*. Metformin has adenosine-monophosphate activated protein kinase (AMPK) -independent effects on LPS-stimulated rat primary microglial cultures. Pharmacol Rep, 2010, **62**(5): 827-848

[3] Labuzek K, Suchy D, Gabryel B, *et al*. Quantification of metformin by the HPLC method in brain regions, cerebrospinal fluid and plasma of rats treated with lipopolysaccharide. Pharmacol Rep, 2010, **62**(5): 956-965

[4] El-Mir M Y, Detaille D, R-Villanueva G, *et al*. Neuroprotective role of antidiabetic drug metformin against apoptotic cell death in primary cortical neurons. J Mol Neurosci, 2008, **34**(1): 77-87

[5] Nath N, Khan M, Paintlia M K, *et al*. Metformin attenuated the autoimmune disease of the central nervous system in animal models of multiple sclerosis. J Immunol, 2009, **182**(12): 8005-8014

[6] Wang J, Weaver I C, Gauthier-Fisher A, *et al*. CBP histone acetyltransferase activity regulates embryonic neural differentiation in the normal and Rubinstein-Taybi syndrome brain. Dev Cell, 2010, **18**(1): 114-125

[7] Ma T C, Buescher J L, Oatis B, *et al*. Metformin therapy in a transgenic mouse model of Huntington's disease. Neurosci Lett, 2007, **411**(2): 98-103

[8] Cabreiro F, Au C, Leung K Y, *et al*. Metformin retards aging in *C*. elegans by altering microbial folate and methionine metabolism. Cell, 2013, **153**(1): 228-239

[9] Fan J, Li D, Chen H S, *et al*. Metformin produces anxiolytic-like effects in rats by facilitating GABAA receptor trafficking to membrane. Br J Pharmacol, 2019, **176**(2): 297-316

[10] Gutierrez A, Khan Z U, Miralles C P, *et al*. GABAA receptor subunit expression changes in the rat cerebellum and cerebral cortex during aging. Brain Res Mol Brain Res, 1997, **45**(1): 59-70

[11] Hartman K N, Pal S K, Burrone J, *et al*. Activity-dependent regulation of inhibitory synaptic transmission in hippocampal neurons. Nat Neurosci, 2006, **9**(5): 642-649

[12] Monier C, Chavane F, Baudot P, *et al*. Orientation and direction selectivity of synaptic inputs in visual cortical neurons: a diversity of combinations produces spike tuning. Neuron, 2003, **37**(4): 663-680

[13] Mcentee W J, Crook T H. Glutamate: its role in learning, memory, and the aging brain. Psychopharmacology (Berl), 1993, **111**(4): 391-401

[14] Markram H, Toledo-Rodriguez M, Wang Y, *et al*. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci, 2004, **5**(10): 793-807

[15] Leventhal A G, Wang Y, Pu M, *et al*. GABA and its agonists improved visual cortical function in senescent monkeys. Science, 2003, **300**(5620): 812-815

[16] Betts L R, Sekuler A B, Bennett P J. The effects of aging on orientation discrimination. Vision Res, 2007, **47**(13): 1769-1780

[17] Memmott R M, Mercado J R, Maier C R, *et al*. Metformin prevents tobacco carcinogen--induced lung tumorigenesis. Cancer Prev Res (Phila), 2010, **3**(9): 1066-1076

[18] Foretz M, Guigas B, Bertrand L, *et al*. Metformin: from mechanisms of action to therapies. Cell Metab, 2014, **20**(6): 953-966

[19] He L, Wondisford F E. Metformin action: concentrations matter. Cell Metab, 2015, **21**(2): 159-162

[20] Marino J, Schummers J, Lyon D C, *et al*. Invariant computations in local cortical networks with balanced excitation and inhibition. Nat Neurosci, 2005, **8**(2): 194-201

[21] Cardin J A, Palmer L A, Contreras D. Stimulus feature selectivity in excitatory and inhibitory neurons in primary visual cortex. J Neurosci, 2007, **27**(39): 10333-10344

[22] Anderson J S, Carandini M, Ferster D. Orientation tuning of input conductance, excitation, and inhibition in cat primary visual cortex. J Neurophysiol, 2000, **84**(2): 909-926

[23] El-Mir M Y, Nogueira V, Fontaine E, *et al*. Dimethylbiguanide inhibits cell respiration *via* an indirect effect targeted on the respiratory chain complex I. J Biol Chem, 2000, **275**(1): 223-228

[24] Burkewitz K, Zhang Y, Mair W B. AMPK at the nexus of energetics and aging. Cell Metab, 2014, **20**(1): 10-25

[25] Manev H, Favaron M, Guidotti A, *et al*. Delayed increase of Ca^{2+} influx elicited by glutamate: role in neuronal death. Mol Pharmacol, 1989, **36**(1): 106-112

[26] Schmolesky M T, Wang Y, Pu M, *et al*. Degradation of stimulus selectivity of visual cortical cells in senescent rhesus monkeys. Nat Neurosci, 2000, **3**(4): 384-390

[27] Riefler G M, Balasingam G, Lucas K G, *et al*. Exocyst complex subunit sec8 binds to postsynaptic density protein-95 (PSD-95): a novel interaction regulated by cypin (cytosolic PSD-95 interactor). Biochem J, 2003, **373**(Pt 1): 49-55

[28] Csermely P, Schnaider T, Soti C, *et al*. The 90-kDa molecular chaperone family: structure, function, and clinical applications. A comprehensive review. Pharmacol Ther, 1998, **79**(2): 129-168

[29] Sheng M, Lee S H. AMPA receptor trafficking and the control of synaptic transmission. Cell, 2001, **105**(7): 825-828

[30] Gerges N Z, Tran I C, Backos D S, *et al*. Independent functions of hsp90 in neurotransmitter release and in the continuous synaptic cycling of AMPA receptors. J Neurosci, 2004, **24**(20): 4758-4766

[31] Ali M M, Roe S M, Vaughan C K, *et al*. Crystal structure of an Hsp90-nucleotide-p23/Sba1 closed chaperone complex. Nature, 2006, **440**(7087): 1013-1017

[32] Prior P, Schmitt B, Grenningloh G, *et al*. Primary structure and alternative splice variants of gephyrin, a putative glycine receptor-tubulin linker protein. Neuron, 1992, **8**(6): 1161-1170

[33] Dumoulin A, Rostaing P, Bedet C, *et al*. Presence of the vesicular inhibitory amino acid transporter in GABAergic and glycinergic synaptic terminal boutons. J Cell Sci, 1999, **112** (Pt 6): 811-823

[34] Farrant M, Nusser Z. Variations on an inhibitory theme: phasic and tonic activation of GABA(A) receptors. Nat Rev Neurosci, 2005, **6**(3): 215-229

[35] Lorenzo L E, Godin A G, Wang F, *et al*. Gephyrin clusters are absent from small diameter primary afferent terminals despite the presence of GABA(A) receptors. J Neurosci, 2014, **34**(24): 8300-8317

[36] Fischer F, Kneussel M, Tintrup H, *et al*. Reduced synaptic clustering of GABA and glycine receptors in the retina of the gephyrin null mutant mouse. J Comp Neurol, 2000, **427**(4): 634-648

[37] Kneussel M, Brandstatter J H, Gasnier B, *et al*. Gephyrin-independent clustering of postsynaptic GABA(A) receptor subtypes. Mol Cell Neurosci, 2001, **17**(6): 973-982

[38] Brunig I, Scotti E, Sidler C, *et al*. Intact sorting, targeting, and clustering of gamma-aminobutyric acid A receptor subtypes in hippocampal neurons *in vitro*. J Comp Neurol, 2002, **443**(1): 43-55

[39] Mcintire S L, Reimer R J, Schuske K, *et al*. Identification and characterization of the vesicular GABA transporter. Nature, 1997, **389**(6653): 870-876

[40] Johnson J, Tian N, Caywood M S, *et al*. Vesicular neurotransmitter transporter expression in developing postnatal rodent retina: GABA and glycine precede glutamate. J Neurosci, 2003, **23**(2): 518-529

[41] Somers D C, Nelson S B, Sur M. An emergent model of orientation selectivity in cat visual cortical simple cells. J Neurosci, 1995, **15**(8): 5448-5465

[42] Dustman R E, Emmerson R Y, Shearer D E. Life span changes in electrophysiological measures of inhibition. Brain Cogn, 1996, **30**(1): 109-126

[43] Carandini M, Heeger D J, Movshon J A. Linearity and normalization in simple cells of the macaque primary visual cortex. J Neurosci, 1997, **17**(21): 8621-8644

二甲双胍调节初级视觉皮层兴奋性和抑制性平衡及改善视觉功能的研究*

蔡　阳　李保炜　周逸峰**

（中国科学技术大学生命科学学院，合肥 230027）

摘要　大脑皮层中兴奋和抑制系统之间的动态平衡决定了皮层神经元对刺激的反应特性. 已有研究表明，二甲双胍能够诱导γ-氨基丁酸受体向突触后膜聚集，增强神经系统的抑制效果. 本课题进一步探讨了二甲双胍对初级视觉皮层兴奋和抑制系统平衡的调节作用，以及其改善小鼠视觉功能的潜力. 实验使用成年雄性小鼠，实验组（metformin）10只每天给予二甲双胍250 mg/kg，对照组（control）6只每天给予0.3 ml生理盐水，灌胃处理3周. 结果发现二甲双胍可以显著升高囊泡GABA转运蛋白VGAT和突触后抑制性递质受体相关蛋白Gephyrin的合成. 此外，它显著降低突触后兴奋性受体GluA1和GluN1的表达. 多通道电极电生理记录结果显示，二甲双胍作用下小鼠初级视觉皮层的自发放和诱发放显著降低，而信噪比、方向和方位选择性显著增加. 实验结果表明，二甲双胍可以通过降低兴奋突触、增强抑制突触，调节初级视皮层的兴奋——抑制平衡，提高信息处理能力，增强视觉功能.

关键词　二甲双胍，初级视觉皮层，多通道电极，兴奋系统，抑制系统

中图分类号　Q5，Q6　　**DOI**：10.16476/j.pibb.2020.0289

* 国家自然科学基金（31571074，91749102）资助项目.

** 通讯联系人.

Tel：13033082368，E-mail：zhouy@ustc.edu.cn

收稿日期：2020-08-07，接受日期：2020-11-02