The Role of KCC2—$GABA_A$ Receptor Converting in the Neuroprotection Induced by Propofol Postconditioning

Authors: Ai Zhu, Hongbai Wang, Shuying Liu, Haiyun Wang

Department of Anesthesiology, Tianjin Research Institute of Anesthesiology, Tianjin Medical University General Hospital, Tianjin 300052, China

Introduction: Our previous studies have proven that propofol postconditioning could induced neuroprotection by regulating the excitatory receptor AMPA receptor[1]. However, the related researches focused on the role of inhibitory receptor $GABA_A$ receptor in the neuroprotection of propofol postconditioning has never been explored. $GABA_A$ receptor is the primary inhibitory receptor in mature mammalian central nervous system, and mediates the flow of chloride ion producing IPSP. KCC2 is a neuron-specific K^+-Cl^- cotransporter that maintains a low intracellular Cl concentration essential for hyperpolarizing inhibition mediated by $GABA_A$ receptors[2]. We tested the the hypothesis that propofol-postconditioning confer neuroprotection by $GABA_A$ receptor, which is regulatd by KCC2 in rat hippocampal slices.

Methods: Organotypic hippocampal slices were subjected to oxygen–glucose deprivation for 7mins, then transferred to the normal ACSF in the presense of propofol 1.2μg/ml for 1h[1]. First, we recorded $GABA_A$ receptor-mediated miniature inhibitory postsynaptic currents (mIPSCs) in vulnerable CA1 pyramidal neurons with whole-cell voltage clamp techniques. To teste whether the protection depended on the changes of $GABA_A$ receptor, bicuculline (100 nmol/L) [2], antagonist of the $GABA_A$ receptor was given with propofol. Meanwhile, cell death was measured with PI. Furthermore, we investigated the regulation of KCC2 on $GABA_A$ receptor via the presence of NEM (agonist of KCC2, 100 μmol/L) with western bolt and messuring the intracellular Cl^- concentration with fluorescence probe.

Results: We found the frequency and amplitude of mIPSCs significantly increased after propofol postconditioning compared with group OGD, and the mortality was also improved, all that was converted by bicuculline. In the presence of NEM, agonist of KCC2, the changes of mIPSCs was also obviously converted like the propofol. Besides, propofol postconditioning reduced the intracellular Cl^- concentration caused by OGD, and up-regulated expression of KCC2.

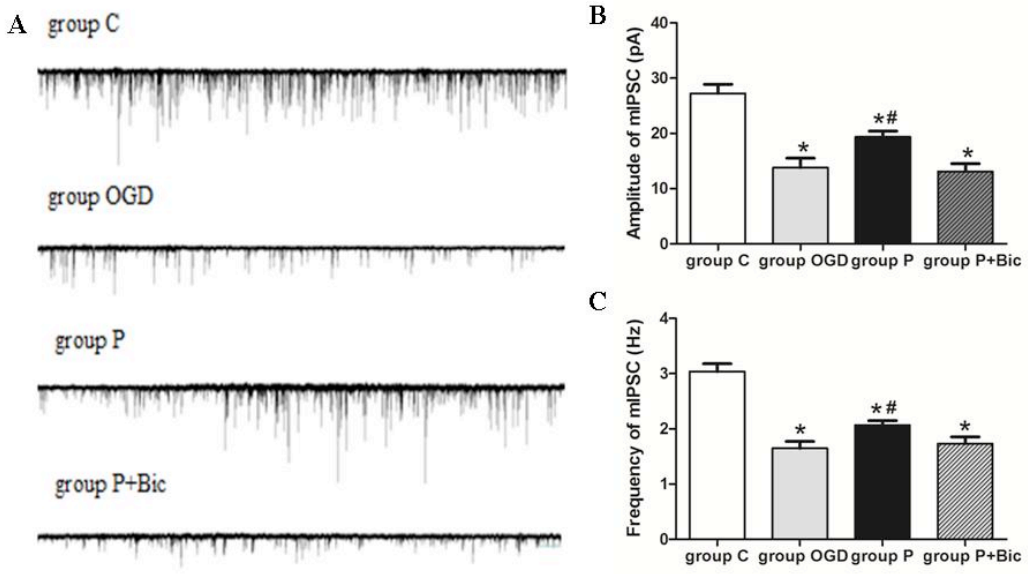
Conclusion: These results suggested that propofol postconditioning could change the flow of Cl^-, which was mediated by KCC2, thus converting the changes of $GABA_A$ receptor induced by OGD. $GABA_A$ receptor could be a potential target for the treatment of ischemia disease. Besides, our further research may concentrate on the crosstalk between the AMPA and $GABA_A$ receptors.

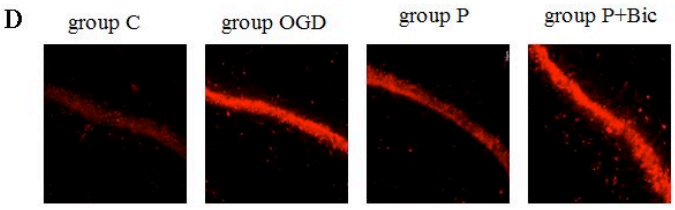
References

1. Wang H，Wang G，Wang C，et al. The Early Stage Formation of PI3K-AMPAR GluR2 Subunit Complex Facilitates the Long Term Neuroprotection Induced by Propofol Post-Conditioning in Rats. PLoS One，2013，8(6)：e65187.

2. DeFazio RA, Raval AP, Lin HW, et al. GABA synapses mediate neuroprotection after ischemic and epsilonPKC preconditioning in rat hippocampal slice cultures. J Cereb Blood Flow Metab, 2009, 29(2): 375-384.

Acknowledgements: This work was supported by Natural Science Foundation of China (81071059, 81100984, 81371245)





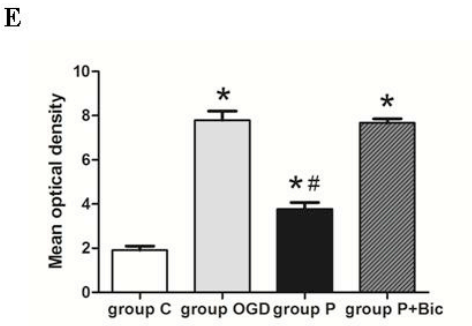
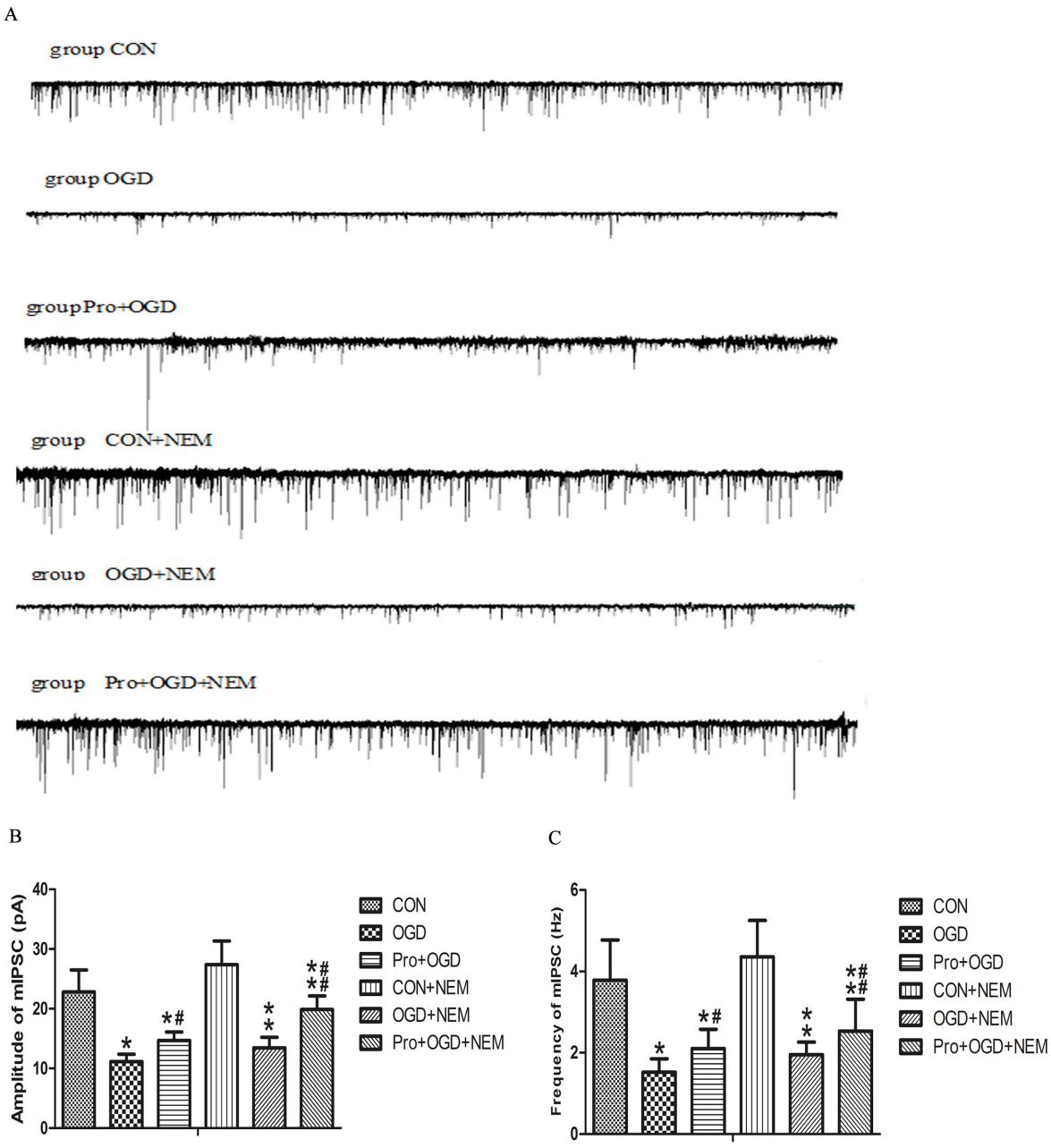


Fig1.$GABA_A$receptor was invovled in the neuroprotection of propofol postconditioning. (A) Representative traces of mIPSC. (B) and (C) Comparision of the amplitude and frequency of mIPSC between each group. (D)and(E) Comparision of the mortality between each group. Error bars represent the mean±SEM.


A
group CON
group OGD
groupPro+OGD
group CON+NEM
group OGD+NEM
group Pro+OGD+NEM
B
Amplitude of mIPSC (pA)
0
10
20
30
40
CON
OGD
Pro+OGD
CON+NEM
OGD+NEM
Pro+OGD+NEM
C
Frequency of mIPSC (Hz)
0
2
4
6
CON
OGD
Pro+OGD
CON+NEM
OGD+NEM
Pro+OGD+NEM

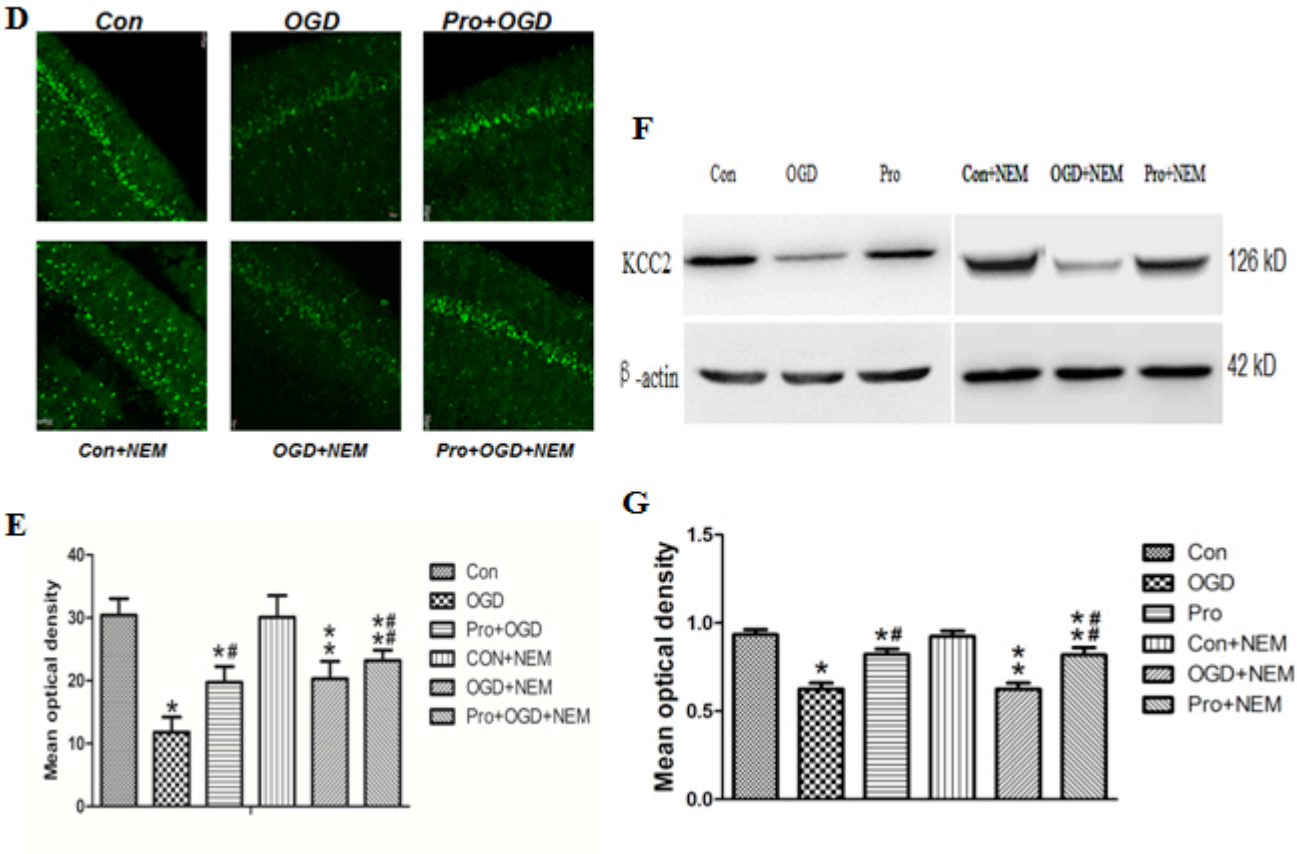


Fig2.GABA$_A$ receptor played its role by the regulation of KCC2.(A) Representative traces of mIPSC.(B) and (C) Comparision of the amplitude and frequency of mIPSC between each group.(D) and (E)Comparision of the concentration of [Cl]_between each group.(F) and (G) Expression and comparision of KCC2 by western blots. Error bars represent the mean±SEM.