The Role of PKA/AKAP in Propofol Post-Conditioning Against Cognitive Dysfunction Induced by Cerebral IR Injury

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Introduction: In our previous investigations, we have confirmed that propofol post-conditioning (20mg/kg/h) provided acute neuroprotection to cerebral ischemia-reperfusion injury in rats via decreasing the internalization of AMPARs receptor GluR2 subunit[1]. However, the effect of propofol post-conditioning to cognitive dysfunction induced by cerebral ischemia-reperfusion and the true mechanism have never been determined. Since the phosphorylation of AMPARs GluR1 can regulate the trafficking of GluR2 and stability of GluR2 lacking AMPARs[2], we focus the experimental research on variation of AMPARs GluR1 and its contributions to cognitive function in rats.

Methods: In this study, we divided rats into 6 groups: shame group, MCAO group, MCAO with propofol (20mg/kg/h) group, and the other three gourps were injected with st-Ht31 (5.5ug, IV) 5 minutes before reperfusion respectively. We firstly evaluated the effect of propofol on nerurological deficit scores and infarct volume using the model of MCAO on rats, while the cognitive function was investigated by fear-conditioning learning test. Furthermore, expression of protein AMPARs GluR1 as well as the phosphorylation level of which were measured by Western blot analysis. We also studied its corresponding upstream regulated protein PKA and AKAP150 through coimmunoprecipitation and Western blot.

Results: We found that neurological deficit scores and infarct volume obviously decreased in post-conditioning group compared with MCAO group. Interestingly, the cognitive function was impaired after 1h ischemia and enhanced by application of propofol post-conditioning, the reinforcement of which lasted for 14 days. The protective effect was expressed through improvement on both hippocampal-dependent and hippocampal-independent memory. At the same time, the phosphorylation of AMPARs GluR1 (Ser 845) was increased in propofol post-conditioning group than MCAO group while the total protein of AMPAR GluR1 were of identical amounts. Furthermore, st-Ht31 reduced neuroprotection and cognitive function of propofol, inhibited phosphorylation of AMPARs GluR1 (Ser 845), downregulated protein level of PKA.

Conclusion: These results suggest that propofol-induced postconditioning can ameliorate brain ischemia reperfusion damage via modulating the phosphorylation of AMPARs GluR1 (Ser845), the protection can be inhibited by

inhibition of PKA/AKAP150 which may be the potential mechanism for acute neuroprotection for cerebral ischemia-reperfusion injury in rats. The results implied that the combination of PKA and AKAP150 is the upstream of AMPARs GluR1 pathway.

Reference:

1. Hai-yun Wang, Guo-lin Wang. 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. Timothy J. Jarome, Janine L. The timing of multiple retrieval events can alter GluR1 phosphorylation and the requirement for protein synthesis in fear memory reconsolidation. Learn Mem. 2012;19:300-6.

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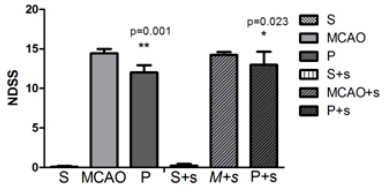

Figure 1. Neurological deficit scoring system score 24h after transient MCAO. Data are expressed as meann ± SEM (n=30). **p< 0.001, compared with the MCAO group. *p< 0.05, compared with the propofol group. The neurological function was significantly improved by propofol post-conditioning, while st-Ht31 partially inversed the function of prpofol.

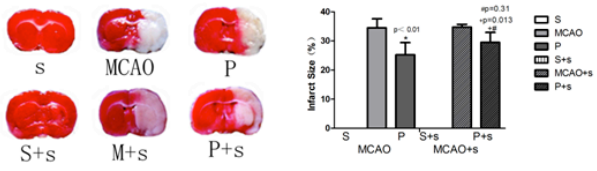

Figure 2. Effect of propofol on infarct volume. Propofol post-conditioning reduced infarct volume, whereas administration of st-Ht31 5min before ischemia partly had no significance compared with propofol group (p=0.31，ANOVA). Data are expressed as mean±SEM(n=6 in each group). *p<0.01 versus MCAO group. +p<0.05 versus MCAO group. #p=0.31 versus propofol group.

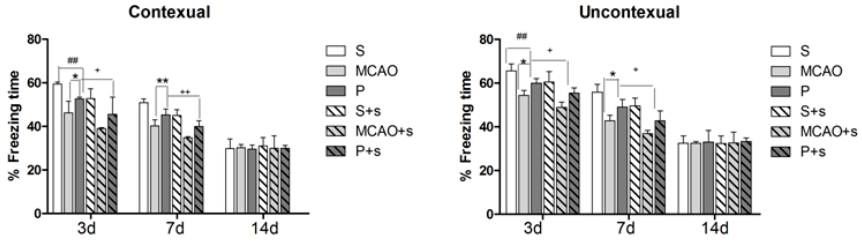

Figure 3. (A) **Hippocampal-dependent memory is protected by propofol post-conditioning and impaired following MCAO or st-Ht31.** It was assessed in separate groups of rats as % freezing time on days 7, 14, 28 after surgery. Values are mean±SEM. On day 7, ##p<0.01 vs shame group, *p<0.05 vs MCAO group, +p<0.05 vs Propofol group; On day 14, **p<0.01 vs shame group, ++p<0.01 vs Propofol group. (B) **Hippocampal-dependent memory is protected by propofol post-conditioning and impaired following MCAO or st-Ht31.**Hippocampal-independent memory was assessed in six groups of rats as % freezing time on 7, 14, 28 after surgery , same as the hippocampal-dependent memory. Values are mean±SEM. On day 7, ##p<0.01 vs shame group, *p<0.05 vs MCAO group, +p<0.05 vs Propofol group; On day 14, *p<0.05 vs shame group, +p<0.05 vs Propofol group.

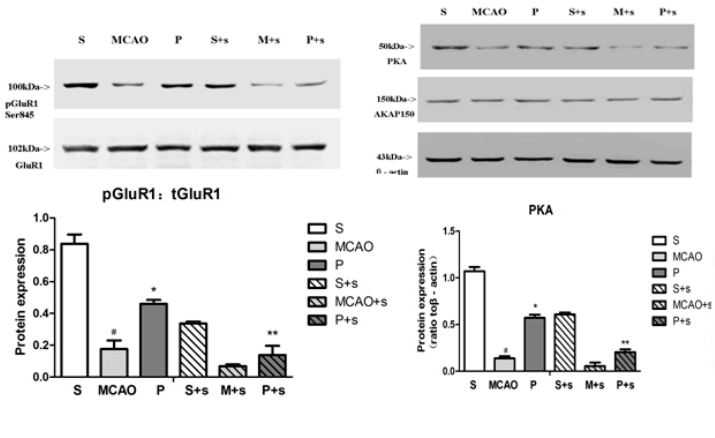

Figure 4: PKA regulate phosphorylation of AMPARs GluR1 in hippocampus tissues of rats.

(4A) Western blot for GluR1, pGluR1(Ser845), PKA, AKAP150 was performed on rat hippocampus.

(4B) The band density was quantitative analysis was measured using Quantity One Analyzer 4.5 (Bio-Rad Laboratories, CA) and analyzed by ANOVO. #p<0.01 versus shame group, * p<0.01 versus MCAO group, **p<0.01 versus Propofol group.

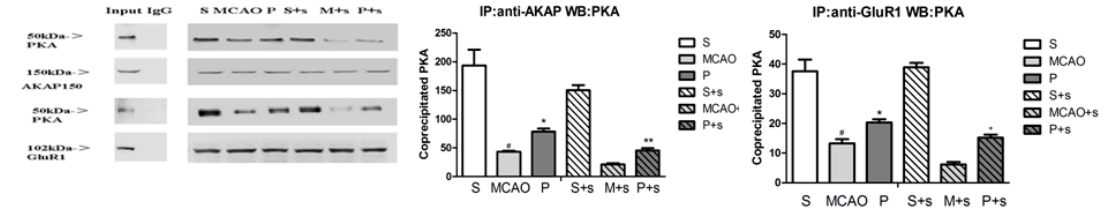

Figure5: PKA was immunoprecipitated with AKAP150 and AMPARs GluR1 in hippocampus tissues of rats.

The content of the precipitate was determined by western analysis. PKA co-located with AKAP 150 and AMAPRs GluR1 in hiappocampus on rats. Ischemia reperfusion injury reduced binding between PKA-AKAP150 and PKA-GluR1. Propofol enhanced the linkage of complexes. The inhibition st-Ht 31 block the link between theses two complexes and inversed the enhancement induced by propofol. Data was described by mean±SEM. #p<0.01 versus to shame group; *p<0.01 versus to MCAO group; ** p<0.01 versus to propofol group; +p<0.05 versus to shame propofol group.