Supplementary information

Activation of GPR35 protects against cerebral ischemia by recruiting monocyte-derived macrophages

Ozayra Sharmin^{1,+}, Ariful Haque Abir^{1,+}, Abdullah Potol^{1,2}, Mahabub Alam¹, Jewel Banik^{1,3}, A.F.M. Towheedur Rahman^{1,4}, Nuzhat Tarannum¹, Rasiqh Wadud^{1,5}, Zaki Farhad Habib^{1,6}, and Dr. Md. Mahbubur Rahman^{1,*}

¹Laboratory of Pharmacology, Department of Pharmaceutical Sciences, North South University, Bashundhra R/A, Dhaka-1229, Bangladesh

Current affiliations:

²Faculty of Medicine, Friedrich Schiller University Jena, 07743 Jena, Germany

³Deptartment of Neurobiology & Developmental Sciences, College of Medicine, UAMS, 4301 W. Markham St., Little Rock, AR 72205, USA

⁴Milwaukee Institute of Drug Discovery, Department of chemistry and Biochemistry, University of Wisconsin-Milwaukee, WI 53211, USA

⁵Department of Veterinary Medicine, University of Cambridge, Madingley Road, Cambridge CB3 0ES, UK

⁶Department of Physiology, Development and Neuroscience, University of Cambridge, Downing Street, Cambridge, UK

Dr. Md. Mahbubur Rahman

SAC1059, Department of Pharmaceutical Sciences,

North South University, Bashundhara R/A, Dhaka-1229, Bangladesh.

Email: rahman.mahbubur@northsouth.edu

^{*}Author of correspondence

⁺These authors contributed equally

Results

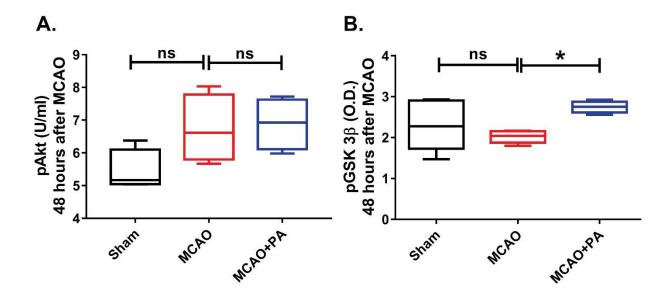


Figure 1: Effect of PA on pAkt and pGSK-3β 48 h after the MCAO.

A. Phospho-Akt concentration was unaffected in the ischemic brain upon pamoic acid treatment at 48 h after the MCAO The One-Way ANOVA, P>0.05 (Bonferroni multiple comparison test), values are means \pm s.e.m, n=4. B. PA treatment significantly increased the phosphorylation of GSK-3 β after 48 h of MCAO. One-Way ANOVA, F(2/13)=4.769, *P=0.0284 (Bonferroni multiple comparison test), values are means \pm s.e.m, n=5.

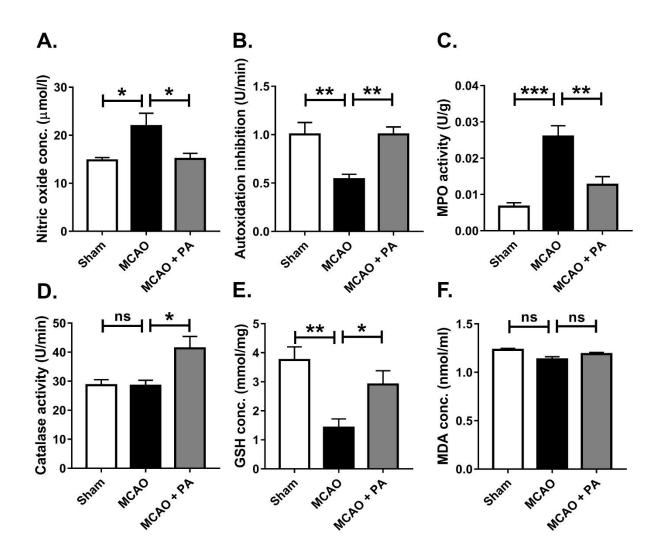


Figure 2: PA treatment reduces oxidative stress in the ischemic hemisphere 48 h after the MCAO.

A. PA treatment reduced the nitric oxide concentration in the ischemic hemisphere 48 h after the MCAO. The One-Way ANOVA, $F_{(2/12)}$ =5.53, *P=0.0364 to 0.0464 (Bonferroni multiple comparison test), values are means±s.e.m, n=5. B. PA treatment increased autoxidation inhibition in the ischemic hemisphere 48 h after the MCAO. The One-Way ANOVA, $F_{(2/12)}$ =10.56, **P=0.0040 to 0.0083 (Bonferroni multiple comparison tests), values are means±s.e.m, (n for Sham =4, n for MCAO= 5, n for MCAO+PA=6). C. PA treatment reduced

myeloperoxidase (MPO) activity in the ischemic hemisphere 48 h after the MCAO. The One-Way ANOVA, $F_{(2/11)}=16.16$, **P=0.0048, ***P=0.0005 (Bonferroni multiple comparison test), values are means±s.e.m,(n for Sham =4, n for MCAO=4, n for MCAO+PA=6).

D. PA treatment increased the catalase activity in the ischemic hemisphere 48 h after the MCAO. The One-Way ANOVA, $F_{(2/12)}$ =6.875, *P=0.0214, (Bonferroni multiple comparison test), values are means±s.e.m, n=5. E. PA treatment increased the GSH activity in the ischemic hemisphere 48 h after the MCAO. The One-Way ANOVA, $F_{(2/17)}$ =9.232, *P=0.0484, **P=0.0019 (Bonferroni multiple comparison test), values are means±s.e.m, (n for Sham =6, n for MCAO=8, n for MCAO+PA=6). F. PA treatment The effect of PA on malondialdehyde (MDA) concentration in the ischemic brain 48 h after the MCAO was insignificant. The One-Way ANOVA, $F_{(2/12)}$ =3.981, P>0.05 (Bonferroni multiple comparison tests), values are means±s.e.m, (n for Sham =4, n for MCAO=5, n for MCAO+PA=6).