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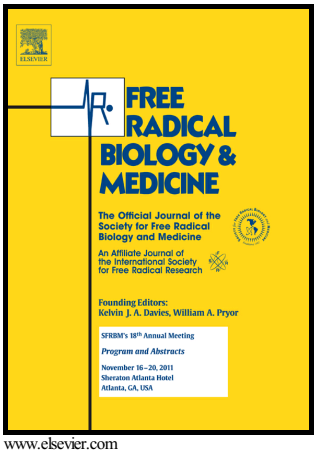

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Hydrogen sulfide reduces RAGE toxicity through inhibition of its dimer formation

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Abstract

RAGE is important in the development of neurodegenerative diseases. The present study was designed to investigate the effect of hydrogen sulfide (H_2S, an endogenous gaseous mediator) on the cytotoxicity caused by RAGE activation during the chronic oxidative stress. $A\beta_{1-42}$ decreased cell viability and induced cell senescence in SH-SY5Y cells. Treatment with advanced glycation end products (AGEs) induced cell injury in HEK293 cells stably expressing RAGE (HEK293-RAGE) and stimulated inflammatory responses in SH-SY5Y cells. Pretreatment of SH-SY5Y cells with an H_2S donor, NaHS, significantly attenuated the above harmful effects caused by $A\beta_{1-42}$ or AGEs. Western blotting analysis shows that oxidative stress enhanced RAGE protein expression which was attenuated by either NaHS or over-expression of cystathionine β-synthase (CBS), a critical enzyme for producing H_2S in brain cells. Both Western blots and split GFP complementation analysis demonstrate that NaHS reduced H_2O_2-enhanced RAGE dimerization. Immunofluorescence analysis shows that H_2O_2 up-regulated the membrane expression of wild-type RAGE. However, H_2O_2–enhanced expression of the RAGE harboring C259S/C310S double mutation (DM-RAGE) was observed in the endoplasmic reticulum. Treatment with NaHS attenuated the effects of H_2O_2 on the protein expression of WT-RAGE, but not that of DM-RAGE. Cycloheximide chase and ubiquitination assays show that NaHS reduced the half-life of WT-RAGE to a similar level of DM-RAGE. S-sulfhydration assay with the tag-switch technique demonstrate that H_2S may directly S-sulfhydrate the C259/C301 residues. Our data suggest that H_2S reduces RAGE dimer formation and impairs its membrane stability. The lowered plasma membrane abundance of RAGE therefore helps to protect cells against various RAGE mediated pathological effects.