論文の内容の要旨

論文題目　Regulation of hydrogen sulfide production and its cytoprotective effect

（硫化水素の生産制御と細胞保護作用の解析）

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Hydrogen sulfide (H_2S) has recently been recognized as a signaling molecule produced in many tissues. H_2S facilitates the induction of hippocampal long-term potentiation (LTP) by enhancing the activity of *N*-methyl D-aspartate (NMDA) receptors in neurons and induces Ca^{2+} influx in astrocytes. Vascular smooth muscle is relaxed by H_2S, which can be released from endothelium as well as smooth muscle. H_2S has pro- and anti-inflammatory effects, nociceptive effects, proangiogenic effect and the regulatory function of insulin release. In addition to a function as a signaling molecule, H_2S can protect cells against oxidative stress. H_2S protects neurons from oxidative stress by reinstating the levels of glutathione (GSH), a major intracellular antioxidant, by enhancing the activity of γ-glutamylcysteine synthetase and the transport of cysteine and cystine. Cardiac muscle is also protected by H_2S from ischemia-reperfusion injury by preserving mitochondrial function.

H_2S is produced by three enzymes, cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and

3-mercaptopyruvate sulfurtransferase (3MST). In the central nervous system, CBS is mainly localized to astrocytes, and 3MST to neurons. 3MST produces H_2S from 3-mercaptopyruvate (3MP), which is generated by cysteine aminotransferase (CAT) from cysteine and α-ketoglutarate (α-KG). However, the cellular distribution and regulation of these enzymes are not well understood.

3MST requires a reducing substance such as dithiothreitol (DTT) to release H_2S. The physiological reducing substance has not been identified. The present study shows that thioredoxin (Trx) and dihydrolipoic acid (DHLA) can release H_2S from persulfide provided by 3MP at the active site of 3MST. Monothiols such as cysteine, GSH and coenzymeA (CoA) as well as the other reducing substances did not show any effect on H_2S-production. 3MST depends on the dithiols, Trx and DHLA, for the production of H_2S from 3MP. H_2S can be produced from thiosulfate in the presence of high concentrations of DHLA. The H_2S production from 3MP and thiosulfate by 3MST is suppressed in the presence of sulfite. These results provide a molecular mechanism for the production of H_2S catalyzed by 3MST.

The present study also shows that 3MST and CAT are localized to retinal neurons and that the production of H_2S is regulated by Ca^{2+}. H_2S can suppress high concentration K^+-induced Ca^{2+} influx in the outer nuclear layer of the retina. Feedback from horizontal cells to photoreceptor cells is mediated

by the suppression of L-type Ca^{2+} channels on photoreceptor cells by protons released from vacuolar type H^+-ATPase (V-ATPase) on horizontal cells. H_2S activates V-ATPase on horizontal cells to release protons that suppress L-type Ca^{2+} channels on photoreceptor cells. Under physiological conditions, H_2S may maintain intracellular Ca^{2+} in low levels. The regulation of Ca^{2+} by H_2S may be failed by the excessive levels of light, and the photoreceptor cell degeneration occurs. The excessive levels of light exposure deteriorated photoreceptor cells and increased the number of terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL)- and 8-hydroxy-2´-deoxyguanosine (8-OHdG)-positive cells. Degeneration was greatly suppressed in the retina of mice administered with NaHS, a donor of H_2S. Even under such conditions the administration of sodium hydrosulfide (NaHS), a donor of H_2S, suppresses light-induced photoreceptor degeneration. These observations suggest that H_2S protects photoreceptor cells from the insult caused by excessive levels of light.