Evidence of Hydrogen Sulfide Involvement in Amyotrophic Lateral Sclerosis

OBJECTIVES: Recently, neuroinflammation has gained a particular focus as a key mechanism of Amyotrophic lateral sclerosis (ALS) [1]. Hydrogen sulfide (H$_2$S) is mainly generated by glial cells in the central nervous system, where it seems to play a double role both as a neuroprotective and as a neurotoxic agent. Infact, it has been demonstrated that H$_2$S exacerbates glutamate-mediated toxicity [2] and raises intracellular calcium into the toxic range in a dose-dependent manner [3]. Our aim is to evaluate the possible role of H$_2$S as a glial-released factor contributing to ALS-mediated motor neuron death.

MATERIALS AND METHODS: H$_2$S concentrations were analyzed in the cerebrospinal fluid (CSF) of 37 sporadic ALS patients and 14 matched controls (Fig.1a), in tissues of a familial ALS (fALS) mouse model (Fig1b), and in spinal cord culture media by means of an innovative high-performance liquid chromatography method (Fig.1c). We analyzed immunohistochemically and by patch clamp recordings and microfluorometry the effects of H$_2$S on motor neurons cultures (Fig.2-3).

RESULTS: We found significantly higher H$_2$S levels in the spinal fluid of ALS patients, with a slight correlation between H$_2$S concentrations and the progression rate of disease. Increased levels of H$_2$S in the tissues and in the media from mice spinal cord cultures bearing the fALS mutation SOD1G93A was also detected. We further show that H$_2$S is mainly released by activated astrocytes and microglia. Moreover, H$_2$S added to spinal culture, obtained from control C57BL/6J mice, is toxic for motor neurons (Fig.2), and induces an intracellular Ca$^{2+}$ increase, attenuated by the intracytoplasmatic application of adenosine triphosphate (ATP) (Fig.3).

DISCUSSION: The presence of a correlation between H$_2$S levels and the progression rate of the disease could suggest a possible prognostic role. The H$_2$S increasing in both human sporadic ALS and in mouse fALS reveals that H$_2$S reaches harmful concentrations in ALS regardless of whether it has a genetic origin. Treating the SOD1G93A cultures with lipopolysaccharide to induce microglia activation, generates an increased production of H$_2$S, suggesting that H$_2$S increasing could be caused by inflammatory processes present in ALS. Furthermore, the attenuation of H$_2$S effects in Ca$^{2+}$ spinal neurons accumulation by addition of ATP, indicates a metabolic failure likely due to a drop of neuronal ATP, by blocking the mitochondrial respiratory chain. Finally, H$_2$S impairs specifically cellular energy production more effectively in an energy-needing cell like the motor neuron.

CONCLUSIONS

H$_2$S can be considered as a new astrogial inflammatory mediator possibly involved in the motor neuron death characterizing ALS.

References