Evidence of Hydrogen Sulfide Involvement in Amyotrophic Lateral Sclerosis

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OBJECTIVES: Recently, neuroinflammation has gained a particular focus as a key mechanism of Amyotrophic lateral sclerosis (ALS) [1]. Hydrogen sulfide (H_2S) 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_2S 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_2S as a glial-released factor contributing to ALS-mediated motor neuron death.

MATERIALS AND METHODS: H_2S 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_2S on motor neurons cultures (Fig.2-3).

Diagnostic group	ALS (total)	ALS BO	ALS ULO	ALS LLO	Controls
Number (N)	37	10	8	19	14
Age (years)*	65.7±10.4	63.6±12.2	60.3±13.3	69.1±6.8	59.2±12.4
Sex (female/male)	16/21	4/6	3/5	9/10	8/6
Disease duration (months)**	9.0 (7.0-12.0)	8.0 (7.0-10.0)	12.0 (9.0-18.5)	10.0 (7.0-12.0)	na
ALSFRS-r score**	40.0 (37.0-44.0)	42.5 (38.0-44.0)	40.0 (37.0-43.5)	40.0 (36.0-44.0)	na
Progression rate**	0.87 (0.44-1.11)	0.64 (0.37-1.11)	0.59 (0.33-0.93)	1.0 (0.5-1.25)	na

	ALS (total) (n=37)	ALS LO (n=27)	ALS BO (n=10)	ALS ULO (n=8)	ALS LLO (n=19)	Controls (n=14)	Significance
H_2S^{csf} (ppm)	7.53 (2.66-13.88)	8.05 (3.12-13.88)	4.84 (2.66-9.24)	6.57 (3.12-12.39)	9.24 (4.60-13.88)	3.64 (0.62-5.70)	ALS(total) > Controls p<0.00001 ALS LO > ALS BO p=0.017 ALS LLO > ALS BO p= 0.003

Fig.1a

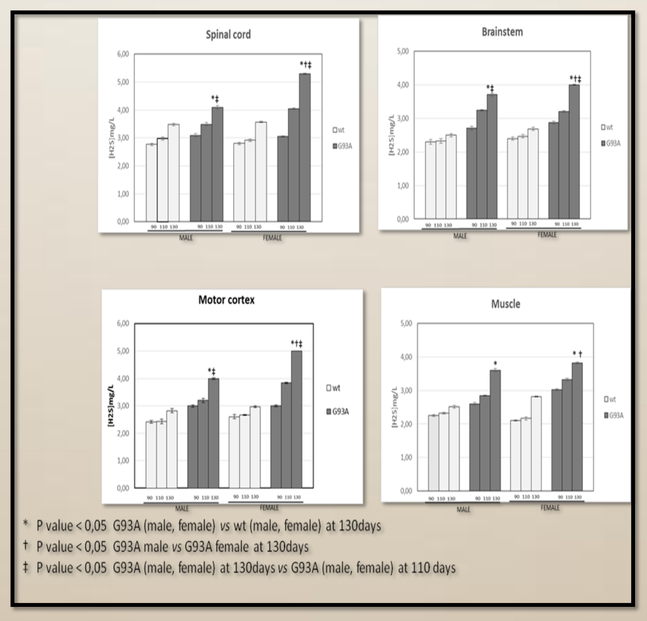

Fig.1b

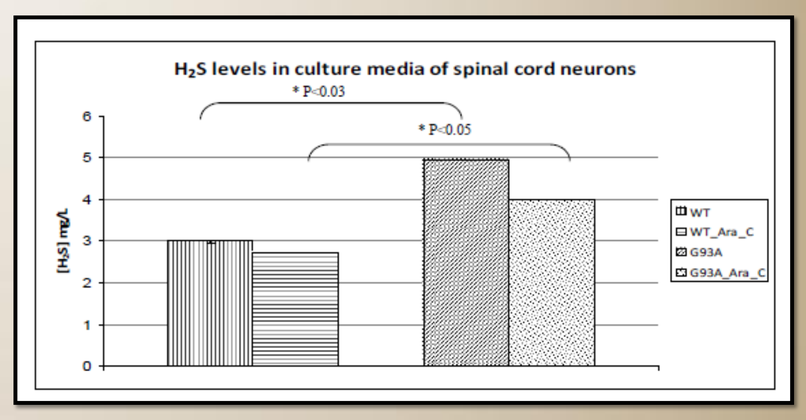

Fig.1c

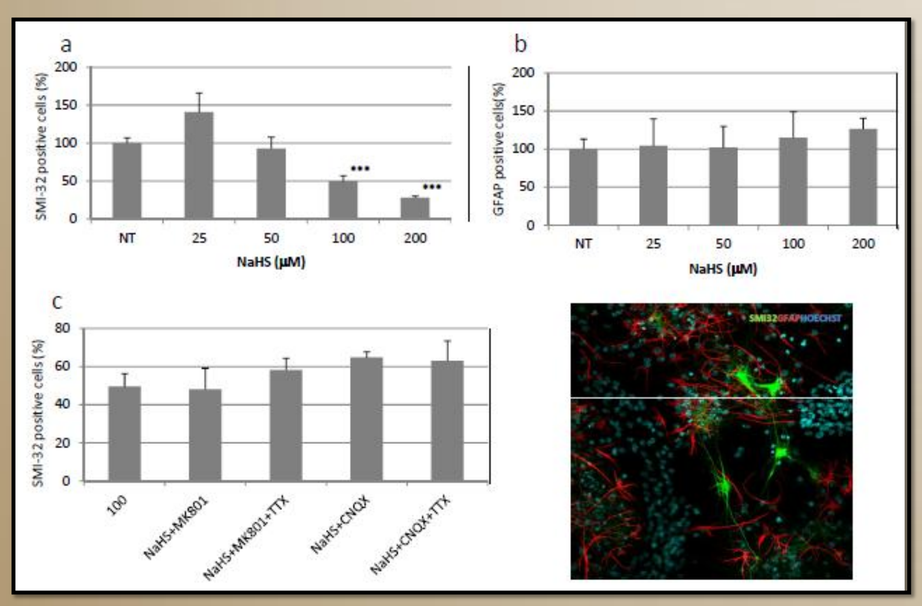

Fig.2

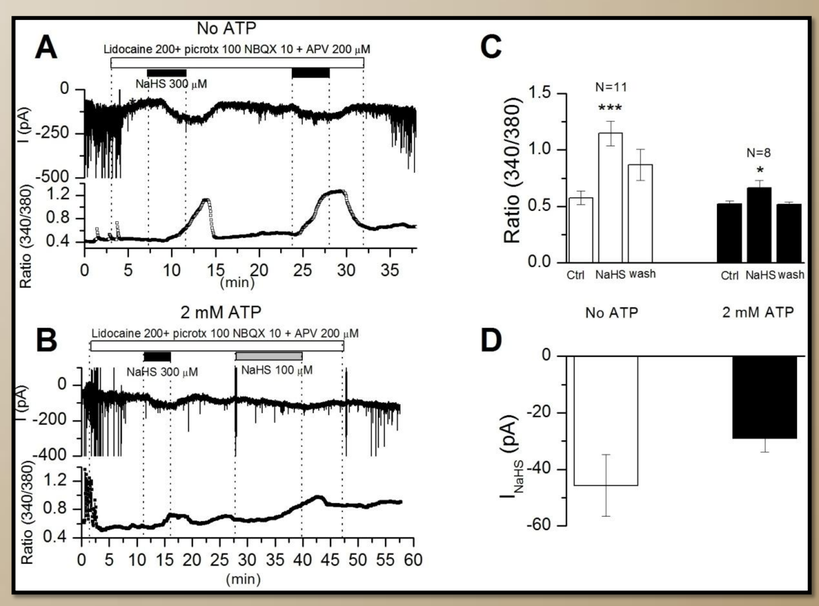

Fig.3

RESULTS: We found significantly higher H_2S levels in the spinal fluid of ALS patients, with a slight correlation between H_2S concentrations and the progression rate of disease. Increased levels of H_2S 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_2S is mainly released by activated astrocytes and microglia. Moreover, H_2S 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_2S levels and the progression rate of the disease could suggest a possible prognostic role. The H_2S increasing in both human sporadic ALS and in mouse fALS reveals that H_2S 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_2S, suggesting that H_2S increasing could be caused by inflammatory processes present in ALS. Furthermore, the attenuation of H_2S 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_2S impairs specifically cellular energy production more effectively in an energy-needing cell like the motor neuron.

CONCLUSIONS

H_2S can be considered as a new astroglial inflammatory mediator possibly involved in the motor neuron death characterizing ALS.

References

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