Hydrogen sulphide is involved in human Malignant Hypertermia

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Background

Malignant hyperthermia (MH) is a pharmacogenetic disorder of skeletal muscle. The incidence of MH reactions ranges from 1:5.000 to 1:50.000 anesthesias, however genetic abnormalities have been estimated as great as one in 3.000 subjects[1]. The symptoms of MH include hyperthermia, increase in carbon dioxide production and oxygen consumption, muscle rigidity, rhabdomyolisis, tachycardia and, if untreated, death. Over 90 mutations have been identified in the ryanodine receptor (RYR1) gene and at least 30 are causal for MH. The genetic test only detects about 30% of people at risk of MH. Therefore, the 'gold standard' for MH diagnosis is the *in vitro contracture test*. These tests enclose a positive response to caffeine, which is a well-known non specific PDE inhibitor[2], and to halothane, whose mechanism of action involves K_{ATP} channels[3]. Since these actions are among the most accredited molecular mechanisms triggered by H_2S[4,5] we aimed to evaluate the role of H_2S/L-Cys pathway in MH.

Methods

The procedure is performed accordingly to the 'European Group protocol for investigation of malignant hyperthermia susceptibly' Briefly, the muscular biopsy of the *vastus* group of the quadriceps muscle is harvested under regional anaesthesia. Eight muscular bundles of 15-25 mm length and 2-3 mm thickness are placed in a tissue bath with Krebs solution at 37 °C and connected to an isometric transducer . Electrical stimulus is than applied and the muscle is stretched slowly up to 2g. After 20 minutes of equilibration caffeine or halothane are added in the tissue bath at progressive concentrations. An increase in resting tension of at least 2 mN allows a MH susceptible (MHS) diagnosis. After diagnosis has been made, muscle bundles from both MHS and MH negative (MHN) subjects have been used for functional studies. Western blot analysis, qRT-PCR for H_2S molecular machinery and plasmatic and tissutal H_2S content were also performed.

Results

H_2S assay performed on tissues homogenate revealed an significant increase of H_2S content in MHS patients compared to MHN. Conversely, no difference was founded in plasmatic levels H_2S of both groups of patients. Western blot analysis showed a significant and selective increase of CBS expression in MHS patients compared to MHN, confirmed by qRT-PCR analysis. Functional studies performed on MHN showed that pre-incubation of the tissue with NaHS was able to switch an MHS typical response following challenge with either caffeine or halothane.

Conclusion

Our data show that in MHS patients there is an increase in CBS expression that accounts for the enhanced concentration of H_2S within the skeletal muscle. The involvement of H_2S in MH is further confirmed by the finding that incubation of MHN tissues with NaHS prior to the addition of either caffeine or halothane generates a response similar to MHS tissues. In conclusion we demonstrate that L-Cys/CBS/H_2S pathway is involved in Malygnant Hyperthermia. This finding may allows a different diagnostic and/or therapeutic approach.

1 Rosenberg et al., 2007
2 Daly et al., 2007
3 Kwok WM et al., 2002
4 Bucci et al., 2010
5 Mustafa et al., 2011