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Bacterial Lipopolysaccharide-Induced Alteration Of Hydrogen Sulphide Synthesis In Porcine Isolated Bronchioles

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Hydrogen sulphide (H_2S) is a naturally occurring mediator of inflammation (Li et al. 2006). It is synthesised in mammalian tissues from L-cysteine by cysthionine-β-synthase (CBS) and cystathionine γ-lyase (CSE) and from 3-mercaptopyruvate by 3-mercaptopyruvate sulfurtransferase (MPST) (Li *et al*.,2011). Previous studies have shown that treatment of hepatocytes for 4 hours with lipopolysaccharide (LPS), a constituent of bacterial cell walls which stimulates an inflammatory response, increases the expression of CSE (Li et al., 2009). We have previously demonstrated that H_2S can be synthesised in isolated porcine bronchioles through the CBS/CSE and MPST pathways (Rashid et al., 2013). The aim of this study was to determine whether treatment of isolated bronchioles with LPS as an in vitro model of airway inflammation, alters the synthesis of H_2S. Segments of small peripheral bronchioles from porcine lungs were dissected and incubated at 37^o C with LPS 1µg/ml in Krebs-Henseleit buffer for 4 hours. Tissues were then homogenised in ice cold Tris-EDTA and cytosolic fractions prepared (Rashid et al., 2013). H_2S synthesis from L-cysteine (10mM) or 3-mercaptopyruvate (0.3mM) was measured in cytosolic fractions of peripheral bronchioles using the methylene blue method (Rashid et al, 2013). CBS, CSE and MPST expression was measured by Western blotting. There was a significant increase in the production of H_2S from 3-mercaptopyruvate in LPS-treated bronchioles compared to control (17.4 ± 2.7 nmoles/mg protein (mean ± s.e.mean), compared to 8.7 ± 2.3 nmoles/mg protein ($p<0.01$ Student's 2-tailed, paired t-test, n=6). However, there was no significant alteration in the production of H_2S through the CBS/CSE pathways (3.6 ± 0.4 nmoles/mg protein (LPS-treated), compared to 3.3 ± 0.4 nmoles/mg protein, n=6). Incubation with LPS had no effect on the protein expression of MPST, CBS, or CSE. These data indicate that there is an enhanced ability to produce H_2S in LPS-treated bronchioles through the MPST enzyme. There was no change in MPST protein expression, suggesting that the increase in H_2S production is likely to be due to an increase in MPST activity. These data indicate that MPST may be important in the airway response to inflammation.

Li et al (2006). *Current Opinion Pharmacol*., 6: 125-129.

Li et al. (2009).*J.Cell.Mol.Med*. 13: 2684-2692.

Li et al. (2011). *Annu. Rev. Pharmacol. Toxicol.*, 51: 169-187.

Rashid et al (2013). *Br. J. Pharmacol.*168: 1902-1910.

Yusuf et al. (2005). *Biochem. Biophys. Res. Commun*., **333**, 1146-1152