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Hydrogen Sulphide Generation in the Heart

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Introduction: Hydrogen sulphide (H2S) has been identified as a signalling molecule in mammals, synthesised by cystathionine β-synthase (CBS), cystathionine γ-lyase (CSE) and 3-mercaptopyruvate sulphurtransferase (MST)[1]. Although there is an abundance of evidence for generation and activity of H2S in vascular tissues1, there is much less information about its role in the heart. The aim of this study was to investigate the expression of H2S-synthesising enzymes in different parts of the myocardium.

Methodology: Pig hearts were dissected into left atrium and left ventricle, quantifying H2S production in cytosols by the methylene blue assay[2]. Expression of CBS, CSE, and MST was determined by immunoblotting[2]. All values were expressed as means ± SEM. Comparisons between more than two data group were made using ANOVA followed by Sidak's post-hoc test. For comparisons between two data sets, a two-tailed unpaired/paired Student's t-test was used. A P-value of less than 0.05 indicated a significant difference between the data set; n= number of animals.

Results: CBS immunoreactivity was detectable in pig atria and ventricle samples at 63 and 48 kDa, with faint bands of CSE at 45 kDa and MST at 30 kDa. Using rat liver cytosol (RLC) as a positive control, the low MW CBS immunoreactivity was more obvious. There were also bands at 63 kDa in the RLC, as well as multiple, fainter, higher MW bands. In the positive control, RLC, L-cysteine or mercaptopyruvate allowed measurable production of methylene blue, with the former blocked completely in the presence of 100 µM AOAA, indicating the activity of CBS and/or CSE. In contrast, preparations from pig heart (either atria or ventricles) failed to generate methylene blue levels which were different from background or inhibited in the presence of 100 µM AOAA, suggesting low levels of enzyme activity in the heart. The additions of DTT (0.5 mM), L-aspartic acid (10 mM), and SAM (2 mM), as well as degassing and adjusting the assay pH to 9 failed to produce significant (P value > 0.05, n= 4-6) changes in enzyme activity (Table 1).

Table 1: The effects of modificatin on enzyme activity

Modification	Control	Modification	n	P value
DTT	3.58 ± 0.61	4.1 ± 0.37	4	NS
Aspartic acid	4.34 ± 0.37	4.12 ± 0.59	4	NS
SAM	4.29 ± 0.45	4.37 ± 0.18	6	NS
degassing	3.61 ± 0.29	3.87 ± 0.38	6	NS
pH 9	2.69 ± 0.44	4.86 ± 0.15	6	<0.05

Conclusion: In summary, although immunoblotting provides evidence for CBS expression, we have

failed to identify parallel H2S production for reasons which are as yet unclear. (1) Dunn et al. (2016) *Pharmacol Ther,* 158: 101-113. (2) Rashid et al. (2013) *Br J Pharmacol,* 168, 1902-1910.