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Development of a SF-7 AM based fluorescence assay for detection of Hydrogen Sulphide in the Porcine Heart

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Introduction: Hydrogen sulphide (H_2S) is mainly synthesised endogenously from L-cysteine through the enzymes Cystathionine β-Synthase (CBS) and Cystathionine γ-lyase (CSE) and from 3-mercaptopyruvate through 3-mercaptopyruvate sulphurtransferase (3-MST)[1]. There is an increasing evidence for generation and activity of H_2S in the vasculature and there is much less information about its role in the heart. The aim of this study was to develop an assay to measure the H_2S-production in the myocardium.

Methodology: Cytosolic fractions of porcine hearts (PHC) were prepared by homogenisation in Tris-EDTA buffer and centrifugation at 30,000 g for one hour. H_2S production through the CBS/CSE pathway was determined using 10mM L-cysteine, whereas H_2S production through the MST pathway was determined using 10 mM mercaptopyruvate as substrate. H_2S generation from these substrates was measured using the methylene blue (MB)[2] assay and compared to that detected using the fluorescent probe sulfidefluor-7 acetoxymethyl ester (SF7-AM) [3]. 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 carried out. A P-value of less than 0.05 indicated a significant difference between the data set; n= number of animals.

Results: The optimal incubation time in the methylene blue assay was 60 minutes for CBS/CSE pathway and 30 minutes for MST pathway while it was 90 minutes for SF7-AM assay in both CBS/CSE and MST pathways. Increasing the pH of the incubation buffer from 7.4 to 9 resulted in an increase in the detection of H_2S production in the CBS/CSE pathway measured by MB and SF7-AM assays. Increasing the pH to 9 caused an increase in H_2S detected through the MST pathway by the MB assay, but not using the SF7-AM assay. Dialysis of the PHC (using semipermeable membrane) resulted in an increase in H_2S detected through the CBS/CSE and MST assays using the SF7 assay (table 1).

Table 1: The effects of modifications on H_2S detection:

Modification	Control	Modification	n	p-value
pH 9 (MB)	5.7±0.5 nmoles/ mg protein	7.6±0.7 nmoles/ mg protein	6	<0.05
pH 9 (SF7-AM/ CBS/CSE)	24.6±2.6 relative fluorescence unit /mg protein	36.63± 3.7 relative fluorescence unit/ mg protein	6	<0.05
pH 9 (SF7-AM/ MST)	32.4±3.3 relative fluorescence unit/ mg protein	37.9±4.5 relative fluorescence unit/ mg protein	6	NS
Dialysis	8.7±1.7 relative fluorescence unit/ mg protein	16.49±2.0 relative fluorescence unit/ mg protein	6	<0.05

Conclusion: In short, SF7-AM can be used to detect H2S production through both the CBS/CSE pathways and the MST pathway in the porcine heart. Dialysis seems to lead to an increase in H_2S detected, which may be due to a removal of an endogenous inhibitor.

References:

(1) Dunn et al., (2016) *Pharmacol Ther*,**158**: 101-113.
(2) Rashid et al., (2013) *British Pharm*, **168**, 1902-1910.
(3) Lin et al., (2013) PNAS, **110** (18), 7131-5.
(4) Yasir et al., (2016) BPS Winter Meeting.