Which Hydrogen Sulphide-Producing Enzyme Mediates Hypoxic Responses In Porcine Coronary Arteries?

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Our preliminary studies have shown a role for hydrogen sulphide in mediating coronary artery vasodilation induced by hypoxia (Garle et al., 2011). The aim of the current study was to expand these findings to investigate which hydrogen sulphide-producing enzyme, cystathionine γ-lyase (CSE) or cystathionine β-synthase (CBS) mediates this response.

Segments of porcine coronary arteries, obtained from a local abattoir, were mounted in organ baths, containing Krebs-Henseleit buffer (37°C) gassed with 95% O₂/5% CO₂, for isometric tension recording. Viability was assessed using 60 mM KCl. Arteries were precontracted with the thromboxane A₂ mimetic, U46619; once a stable tone (~50% of KCl response) was achieved hypoxia was induced using 95% N₂/5% CO₂ for 30 min. Hypoxic responses were investigated in the absence and presence of inhibitors of CSE and/or CBS. Data were presented as mean ± SEM with contractions expressed as a percentage of the response to KCl, relaxations expressed as a percentage of the U46619-induced tone or as area under the curve (AUC). Data were analysed using Student’s t test or 2-way ANOVA with Bonferroni post-test where P<0.05 is considered significant (n=8).

Exposing coronary arteries to hypoxia caused a biphasic response, a transient contraction followed by a prolonged relaxation. The CSE inhibitor D,L-propargylglycine (PPG) alone (10, 30 or 100 µM) had no effect on the vascular response to hypoxia. In contrast, the CBS inhibitor amino-oxyacetate (AOAA) alone (0.1, 0.3 or 1 mM) caused a significant reduction in the contraction at 100 µM (control 14 ± 2%, +AOAA 10 ± 3%, P=0.02) and inhibited the relaxation at higher concentrations (e.g.1 mM control 60 ± 4%, +AOAA 34 ± 5%, P=0.008).

When PPG and AOAA were applied in combination, they caused a significant inhibition of both the contraction and relaxation phases and produced a greater effect than either alone e.g. 10 µM PPG and 100 µM AOAA had no effect on relaxation alone but when applied together they caused a significant reduction in relaxation (e.g. Control AUC 2839 ± 368, +PPG/AOAA AUC 1713 ± 377 P=0.03). AOAA alone and PPG/AOAA in combination had no effect on the concentration-response profile of the K_ATP channel opener pinacidil, with the exception of 30 µM/300 µM (R_max control 131 ± 9, +PPG/AOAA 114 ± 4, P=0.02).

These data show that H₂S is an endogenous mediator of the hypoxic response of the porcine coronary artery. It was surprising to find that PPG had no effect when applied alone on the response to hypoxia given that CSE is widely expressed throughout the cardiovascular system. However, when the enzyme inhibitors were applied in combination there was a greater inhibition suggesting that complex mechanisms are involved which require further investigation.

Garle, M et al. (2011) www.pA2online.org/abstracts/Vol9Issue3abst099P.pdf