Proceedings of the British Pharmacological Society at http://www.pA2online.org/abstracts/Vol10Issue4abst068P.pdf

Enhanced synthesis of hydrogen sulphide in liver and kidney from Zucker diabetic rats compared to Wistar rats.

Samia Rashid, Steve Alexander, Richard Roberts. University of Nottingham, Nottingham, UK

Hydrogen sulphide (H_2S) is a gasotransmitter that has a number of biological functions, including a role in inflammation and vasodilatation (Li *et al*, 2011). H_2S is synthesised in mammalian tissue from L-cysteine by two pyridoxal-5-phosphate dependent enzymes; cysthionine-β-synthase (CBS) and cystathionine γ-lyase (CSE) (Li *et al*.,2011). Alternatively, H_2S can be produced from 3-mercaptopyruvate by 3-mercaptopyruvate sulfurtransferase (MPST). The aim of this study was to determine whether the synthesis of H_2S is altered in Zucker diabetic rats.

Brains, livers, kidneys and lungs were dissection out of 12-13 week-old male, Zucker diabetic rats (mean non-fasting blood glucose levels 26 mM, mean weight 390g). Male Wistar rats (mean weight 300g) were used as a non-diabetic control. H_2S synthesis from L-cysteine (10mM) or 3-mercaptopyruvate (0.3mM) was measured in cytosolic fractions of each tissue using the methylene blue method (Stipanuk and Beck, 1982). Generation of methylene blue was detected by measuring absorbance at 670 nm.

There was a significant increase in the production of H_2S from L-cysteine in kidneys from diabetic rats compared to Wistar rats (10.9 ± 1.5 nmol/mg protein (mean ± s.e.mean) in Zuckers v 6.2 ± 0.5 nmol/mg protein in Wistars, $p<0.05$, 2-tailed, unpaired t-test, n=4), but no significant effect in other tissues. H_2S production from 3-mercaptopyruvate was increased in kidney (69.2 ± 7.4 nmol/mg protein in Zuckers v 33.0 ± 4.3 nmol/mg protein in Wistars, $p<0.01$, 2-tailed, unpaired t-test, n=4) and liver (192.3 ± 10.5 nmol/mg protein in Zuckers v 130.8 ± 12.1 nmol/mg protein in Wistars, $p<0.01$, 2-tailed, unpaired t-test, n=6) from diabetic rats, but there was no significant change in brain and lungs. Although there was no change in the total level of H_2S synthesis in the brain, the CSE inhibitor propargylglycine (10µM) reduced the response from 3.3 ± 0.5 nmol/ mg protein to 0.06 ± 0.003 nmol/mg protein in Wistar rats ($p<0.001$, 1-way ANOVA followed by a Dunnett's post-hoc test, n=4), but had no effect in Zucker rats, suggesting that there may be an alteration in the relative expression H_2S synthesising enzymes. These data indicate that there is an enhanced ability to produce H_2S in kidneys and livers from diabetic rats. Similar changes have been observed by others in pancreatic β-cells from Zucker diabetic rats, and liver from streptozotocin-diabetic rats (Wu *et al*., 2009; Yusuf *et al*., 2005). Whether these changes in H_2S production play a role in the pathogenesis of diabetes, or are a protective effect remains the subject of future investigations.

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

Stipank et al.(1982).*Journal of Biochem, 206,* 267-77,

Wu et al., (2009). *Laboratory Investigation*, **89**, 58-67.

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