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Infarct Limitation by GYY4137 at Reperfusion, a Slow-Releasing Hydrogen Sulfide Donor, in the Rat *In Vivo*

Administration of exogenous hydrogen sulfide (H_2S) donors has been shown to protect the heart against ischaemia/reperfusion injury when given at reperfusion by a mechanism that is not fully defined. We sought to characterise the effect of GYY4137, a slow-releasing H_2S donor (morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate), when given specifically at reperfusion and the involvement of PI3K/Akt signalling in mediating its effects. . All the handling and procedures were carried out in accordance with Home Office Guideline of the Animal (Scientific Procedure) ACT, 1986. Adult male Sprague Dawley rats were anaesthetised with thiobutabarbital (800 μmol/kg, i.p.) and regional myocardial ischaemia was induced by occluding the left descending coronary artery for 30 minutes followed by 2 hours of reperfusion. Animals were randomly assigned (n=6 per group) to receive: 1) no further intervention, 2) GYY4137 (266 μmol/kg, i.v.) 10 minutes before reperfusion, 3) Endothelial nitric oxide synthase (eNOS) inhibitor (L-NAME, 74.2 μmol/kg, i.v.) 5 minutes prior to GYY4137 dose, 4) PI3K inhibitor LY294002 (872.6 nmol/kg, i.v.) 5 minutes prior to GYY4137 dose. In two additional groups, L-NAME and LY294002 were given alone as previously. In a parallel series of experiments (n-4 in per group), tissue samples were harvested from the left ventricle at 5 minutes of reperfusion and snap frozen for biochemical analysis. Infarct size is reported as a percentage of the area at risk. Data, reported as mean±SEM, were analysed using one-way ANOVA supported by Newman Keuls post hoc test, when appropriate. In GYY4137-treated hearts, the phosphorylation of Akt, eNOS and GSK-3β increased at 5 minutes of reperfusion. GYY4137 significantly limited infarct size by 51% compared to untreated-hearts (27.6 ± 2.0% vs 56.8 ± 3.5%, $p<0.001$). Co-administration of L-NAME with GYY4137 attenuated but did not abolish the cardio protection established by GYY4137 (41.1 ± 6.3% vs 27.6 ± 2.0%, $p<0.05$) by limiting the phosphorylation of eNOS. LY294002 totally abrogated the infarct-limiting effect of GYY4137 (55.7 ± 3.3%) and inhibited the phosphorylation of Akt, eNOS and GSK-3β. These data indicate that GYY4137 can protect the heart against ischaemia/reperfusion injury through activating the PI3K/Akt/NO signalling pathway and increasing the phosphorylation of GSK-3β at early reperfusion.