FUNCTIONAL ANALYSIS OF PUTATIVE CARDIO-PROTECTIVE AGENTS IN REPERFUSION OF WHOLE RAT HEART AND ISOLATED RAT CARDIOMYOCYTES

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Declaration

I, Amer Hasan Almashhadany certify that the substance of this thesis has not already been submitted for any degree and is not being currently submitted for any other degree.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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Abstract

The amino acids, glycine and glutamine have been implicated in myocardial protection against damage which occurs when the ischaemic heart is reperfused. A major focus of this thesis was to investigate whether such protection could be enhanced by simultaneously delivering these amino acids as the dipeptide, L-glycyl-L-glutamine (gly-gln). In addition homocysteine has been implicated in augmented oxidative stress, and recognized as a risk factor for developing cardiovascular disease, but is structurally related to the cardioprotective agent cysteine. A second aim, therefore, was to investigate the little known effects of homocysteine on heart cells. Excised hearts from male Wistar rats were perfused in the Langendorff mode. For measurements of functional performance and reperfusion damage the perfusion protocol comprised 20 minutes baseline perfusion, 40 minutes global normothermic ischaemia (no perfusion) followed by 40 minutes reperfusion with Krebs solution (all at 37 ℃ in the Langendorff system). Where present 0.5, 2 and 5 mM gly-gln was added 10 minutes into baseline perfusion, was present throughout ischaemia and was washed out after

10 minutes reperfusion. In separate experiments small samples of the left ventricle were collected at the beginning and end of the 40 minute ischaemic period and analysed for lactate concentration. Small samples of the right ventricle were collected at the end of the ischaemic period for measurement of thiobarbituric acid reactive substances (TBARS). In the second study, cardiomyocytes were isolated from hearts excised from male Wistar rats using three different approaches: Tyrode buffer alone, Tyrode with 40mM taurine added and Tyrode with glutamate and carnitine added. The success of each approach was analysed by comparing the cellular morphology and viability during exposure to oxidative stress in the presence of 0.05 mM homocysteine. At the same time and in the same experiments the effect of homocysteine in the presence or absence of oxidative stress was investigated. In all studies data presented are means ± SE of n= 4-7 and compared using ANOVA with a Tukey post-test.

The presence of 2 mM gly-gln significantly improved the recovery of left ventricular developed pressure in hearts isolated from 36 week old rats from 18.49 ± 3.01 to 33.9 ± 4.85 mm Hg ($p < 0.02$) and lengthened the time to ischaemic

contracture from 14.02 ± 1.4 to 23.63 ± 1.63 minutes ($p < 0.01$) compared to control. In the second set of experiments the percentage of viable cells isolated in Tyrode was 64.4% ± 1.8 (mean ± SD) which was significantly less than the 80.7% ± 2.4 (mean ± SD) in Tyrode solution containing taurine and the 80.3% ± 1.9 (mean ± SD) in Tyrode with added carnitine and glutamate. After 210 minutes incubation at 37°C there were more viable cells in cells isolated in the presence of Tyrode with taurine compared to the other two isolation methods (55.1% rod shape cells in control, 42.6% rod shape cells in homocysteine treatment, 31.3% rod shape cells in H_2O_2 treatment, 20.1% rod shape cells in homocysteine+ H_2O_2 treatment). In all groups the percentage viability and percentage of rod shaped cells decreased in the presence of H_2O_2, homocysteine and H_2O_2 plus homocysteine, however by far the greatest decline was seen with H_2O_2 plus homocysteine (20.1% rod shape cells in Tyrode solution containing added taurine by comparison with 4.8% rod shape cells in Tyrode alone). Overall, the results of the two studies in this thesis suggest that: 1), Gly-gln shows good potential as a combatant against ischaemia reperfusion injury in middle aged rat hearts; and 2), cells isolated in the presence of taurine over time showed higher viability

compared to cells isolated in Tyrode alone or Tyrode containing glutamate and carnitine. Furthermore, although homocysteine by itself did not affect the isolated cardiomyocytes, in the presence of oxidative stress simultaneous homocysteine exposure worsened cell outcomes compared to oxidative stress alone. These studies provide supporting evidence to the hypothesis that homocysteine augments oxidative stress.

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Publication arising from this thesis

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Abbreviations:

$MgSO_4$	Magnesium sulfate
MDA	Malondialdehyde
β-NAD	β-nicotinamide adenine dinucleotide
PCA	Perchloric acid
KCl	Potassium chloride
ROS	Reactive oxygen species
NaCl	Sodium chloride
AAPH	2,2-Azobis(2-methylpropionamidine) dihydrochloride
NaOH	Sodium hydroxide
NaH_2PO_4	Sodium dihydrogen phosphate
TCA	Trichloroacetic acid
TBA	Thiobarbituric acid

AAPH	2,2-Azobis(2-methylpropionamidine) dihydrochloride
$CaCl_2$	Calcium chloride
EGTA	Ethylene glycol-bis(2- aminoethylether)-*N,N,N′,N′*-tetraacetic acid
Gln	Glutamine
Gly	Glycine
$MgCl_2$	Magnesium chloride
TBARS	Thiobarbituric acid reactive substances
HEPES	4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid)