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MOLECULAR MECHANISMS OF ENCHANCED [18F] FLUORODEOXY GLUCOSE (FDG) UPTAKE IN ISCHEMICALLY INJURED MYOCARDIUM: THE ROLE OF GLUCOSE TRANSPORTER AND HEXOKINASE EXPRESSION

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The specific aims for the original proposal are listed below. The relevant portion of the progress report immediately follows each specific aim.

Aim 1. To determine the relative expression of myocardial glucose transporters in normal and ischemic dog myocardium, and to correlate this expression to glucose uptake and phosphorylation as determined by ^{18}F-fluorodeoxyglucose (FDG) uptake.

Results: We determined that there were no regional differences in GLUT1 or GLUT4 expression in normal dog heart. Then we demonstrated that glucose uptake was relatively enhanced in regions of severe ischemia in this model. Finally we showed that GLUT1 mRNA and polypeptide expression but not GLUT4 expression were substantially and significantly increased in both ischemic and nonischemic myocardial regions after 6 hours (J Mol Cell Cardiol, 1997; 29:1675-1685).

We also found that GLUT4 translocation and glucose uptake induced by ischemia in perfused rat hearts were not inhibited by Wortmannin, a PI3 kinase inhibitor, whereas insulin-stimulated increases in GLUT4 trasnlocation and glucose uptake were inhibited. This suggests that ischemia induces GLUT4 translocation in heart through a different signaling pathway than does insulin (J Cardiovasc Res, 1997; 35:283-293).

Aim 4. To determine, through the use of quantitative reverse transcription-polymerase chain reaction (RT-PCR) techniques, the relative GLUT4 and HK mRNA levels from chronically ischemic and non-ischemic regions of humans with coronary heart disease and to determine, through semi-quantitative immunohistochemical techniques, the expression of GLUT4 and HK proteins. These findings will then be correlated to regional glucose uptake and phosphorylation as determined by ^{18}F-fluorodeoxyglucose (FDG) PET studies.

Results: To determine whether some of the same phenomena occurred in humans with chronic myocardial ischemia, we investigated myocardial GLUT mRNA expression in 11 patients who underwent coronary artery bypass surgery. Before surgery, positron emission tomographic-fluorodeoxyglucose (FDG) scanning was performed which characterized heart regions as normal (N)normal glucose uptake and normal blood flow) , or hibernating (H) (reduced blood flow; increased FDG uptake). Biopsies from these regions were subjected to RT-PCR analysis for GLUT1 and GLUT4 expression. GLUT1 (normalized to cyclophilin) mRNA levels were higher in the H vs. the N regions whereas GLUT4 mRNA levels were not significantly increased. These findings suggest that in humans, chronically ischemic ("hibernating") myocardium expresses higher levels of GLUT1 mRNA than does non-ischemic myocardium from the same heart. These results did not address the issue of whether ischemia increases GLUT1 (or GLUT4) expression in normal regions of these chronically ischemic hearts as it did in dog hearts with persistent ischemia. (Am J Cardiol, 1997; 80:77A-84A).

Aim 5. To establish a cell culture system to test the factors which may be responsible for altering GLUT1 mRNA and protein expression and to determine whether and how GLUT1 expression prevents hypoxia-induced cell death.

We have cultured neonatal rat cardiomyocytes and tested the effects of several factors including hypoxia and insulin. Exposure to 100 nM insulin for 24 hours increased GLUT1 mRNA expression by 1.7 ± 0.3-fold compared to cells not treated with insulin. This suggests that chronic exposure to insulin will increase sarcolemmal GLUT1 expression and that hypoinsulinemia may decrease GLUT1 expression. We also studied the effects of hypoxia on GLUT1 mRNA expression and LDH release. Cells exposed to FiO_2 of ca. 40 mmHg for 1 and 3 hours increased GLUT1 levels to 1.5 ± 0.02 and 1.5 ± 0.1 vs 0.95 ± 0.24 for control cells. Thus, although insulin and moderate hypoxia increase GLUT1 mRNA levels, neither increases GLUT1 levels to the same extent as does ischemia in the dog heart model. This suggests that other mechanisms exist by which ischemia further induces GLUT1. One such factor is adrenergic stimulation. We have found in preliminary studies that isoproteronol (10^{-5} M) increases GLUT1 at least 2-fold. Other candidates include TNF which is released into the coronary circulation in myocardial ischemia and has been shown to induce several signal transduction pathways including the cJun-NH_2-terminal kinase kinase cascade.

In order to test whether increasing the number of glucose transporters on the plasma membrane of cells could elicit a similar protective response, independent of the levels of extracellular glucose, we overexpressed the facilitative glucose transporter, GLUT1 in a vascular smooth muscle cell line. After 4 hours of hypoxia, the percentage of cells which showed morphologic changes of apoptosis was 30.5±2.6% in control cells and only 6.0±1.1% and 3.9±0.3% in GLUT1-overexpressing cells. Similar protection against cell death and apoptosis was seen in GLUT1-overexpressing cells treated for 6 hours with the electron transport inhibitor, rotenone. In addition, hypoxia and rotenone stimulated (JNK) activity >10-fold in control cell lines, and this activation was markedly reduced in GLUT1-overexpressing cell lines. A catalytically-inactive mutant of MEKK1, an upstream kinase in the JNK pathway, reduced hypoxia-induced apoptosis by 39%. These findings show that GLUT1 overexpression prevents hypoxia-induced apoptosis possibly via inhibition of stress-activated protein kinase pathway activation (submitted).