

Public Abstract

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Title:MECHANISMS OF ADENOSINE MONOPHOSPHATE-ACTIVATED PROTEIN KINASE-INDUCED PRECONDITIONING IN ISCHEMIA/REPERFUSION

The phenomenon of ischemic preconditioning (IPC) to protect against the sequelae of ischemia/reperfusion (I/R) as seen clinically with heart attacks, strokes, and transplant surgeries, has received increasing attention since it was first discovered that brief bouts of ischemia and reperfusion prior to the prolonged ischemic insult paradoxically provides protection. Unfortunately, clinical trials aimed at harnessing this innate protective mechanism with various preconditioning stimuli have produced disappointing results. As the signaling mechanisms involved in the development of a protective phenotype are further elucidated, more specific and effective pharmacologic means can be developed to target key initiators, mediators, or effectors to elicit these salubrious events. One such potential preconditioning pathway is initiated by low dose ethanol consumption. We have shown previously that consuming the ethanol equivalent of one to two alcoholic beverages twenty-four hrs prior to a prolonged ischemic challenge (ethanol preconditioning, EPC), protects mice against I/R injury following forty-five min of ischemia and sixty min of reperfusion. We hypothesized that the serine/threonine kinase, adenosine monophosphate-activated protein kinase (AMPK), is activated by ethanol and in turn leads to endothelial nitric oxide synthase (eNOS) phosphorylation. The activation of eNOS to increase nitric oxide (NO) production has many beneficial effects both directly and indirectly. Here we show that AMPK activation by preconditioning with either ethanol or with direct pharmacologic activation via 5-Aminoimidazole-4-carboxamide 1- β -D-ribofuranoside (AICAR), twenty-four hrs prior to the prolonged I/R (EPC and AICAR-PC, respectively), significantly reduced the number of rolling and adherent leukocytes in the post-capillary venules of the mouse small intestine as quantified using intra-vital microscopy. Ten single, unbranched venules with external diameters ranging from twenty-five to fifty microns were recorded for one minute each, following thirty and sixty min of reperfusion. Leukocytes were labeled with 5-(and-6)-carboxyfluorescein diacetate, succinimidyl ester (CFDASE), and determined to roll based on flow rates significantly below the mean centerline velocity of non-rolling leukocytes, and to be adherent if they remained stationary for a minimum of thirty sec. These leukocyte-endothelial cell adhesive interactions (LEI) were used as an index of the severity of the I/R injury, as they are some of the earliest and most sensitive indicators of this inflammatory response. Several other proteins in addition to AMPK and eNOS were also found to be involved in the protective signaling pathway, including both surface (or plasmalemmal) and mitochondrial adenosine triphosphate-sensitive potassium channels (sKATP and mKATP, respectively) and heme oxygenase (HO). Thus our overall hypothesis that EPC requires AMPK activation, and AMPK activation via AICAR-PC requires eNOS, KATP channels, and HO to induce the protective phenotype against postischemic leukocyte rolling and adhesion in postcapillary venules of the murine small intestine was proven to be true. By further identifying the participating proteins and signaling molecules involved in this complex event, the potential for therapeutically relevant targets can be examined and ultimately utilized to reduce the morbidity and mortality associated with some of the leading causes of death in the modern world.