

10th ANNUAL DEPARTMENT OF PATHOLOGY YOUNG INVESTIGATORS' DAY
POSTER SESSION

POSTER #

13

(for Admin. use)

Thursday, April 17th, 2008
TURNER CONCOURSE
REGISTRATION FORM

Applicant's Name: _____ Samarjit Das _____ Degree: Ph.D.

Applicant's Division: _____ Cardiac-Pathology _____

Faculty Preceptor: _____ Charles Steenbergen _____
(Must hold a primary appointment in Pathology)

Appointment Category: _____ House Staff _____ Clin Fellow X _____ Research Fellow
_____ Medical Student _____ Graduate Student (Program: _____)

Register for: _____ Clinical Research _____ Translational Research X _____ Basic Research

Full Poster Title * **THE CARDIOPROTECTIVE EFFECT OF GLYCOGEN SYNTHASE KINASE-3 β (GSK-3 β) INHIBITORS INVOLVES INHIBITION OF MITOCHONDRIAL ADENINE NUCLEOTIDE TRANSPORT**

Where has the work been presented?

Meeting Name American Heart Association Committee on Scientific Sessions Program

Meeting Date November 6, 2007

Not Previously Presented N.A.

Where is this work being published? N.A.

Journal Name, Volume, Page, Date N.A.

In Preparation (Y/N) - Where? N.A.

Author(s) (First & Last) Samarjit Das and Charles Steenbergen

In-House Address: Ross Research Building, Room # 632

(Room # and Building Name, Lab, etc.)

Telephone: (410) 502 6921 _____ Beeper: N.A.

Fax: (410) 502 5862 _____ E-mail: sdas11@jhmi.edu

***INCLUDE A ONE-PAGE ABSTRACT (including title and all authors) OF THE WORK YOU WILL BE PRESENTING**

E-mail COMPLETED Registration form and abstract to:

Stacey Morgan (smorgan9@jhmi.edu) on or before

Friday, March 14th, 2008

If you have questions or problems regarding your submission, please contact Stacey Morgan via e-mail (smorgan9@jhmi.edu)

THE CARDIOPROTECTIVE EFFECT OF GLYCOGEN SYNTHASE KINASE-3 β (GSK-3 β) INHIBITORS INVOLVES INHIBITION OF MITOCHONDRIAL ADENINE NUCLEOTIDE TRANSPORT

Samarjit Das¹, Elizabeth Murphy² and Charles Steenbergen¹

¹Department of Pathology, Johns Hopkins University, Baltimore, MD 21205, USA

²Cardiac Physiology Section, Vascular Medicine Branch, NHLBI, Bethesda, MD 20892, USA

Inhibition of GSK-3 β has been shown to reduce ischemia-reperfusion injury by mechanisms that involve the mitochondria. We aimed to determine the targets of this cardioprotective effect. In rats, 15 min treatment with specific GSK-3 β inhibitors prior to 20 min of ischemia, significantly improved post-ischemic left ventricular function compared to control. Mitochondria were isolated immediately after the perfusion with/without GSK-3 β inhibitors. GSK-3 β inhibitors slightly reduced ADP-stimulated (state 3) respiration and significantly reduced ATP consumption during anoxia. Anoxic ATP consumption was measured by allowing mitochondria in an oxygraph chamber to consume oxygen until they became anoxic, and the rate of consumption of added ATP was measured at 20, 40, and 60 minutes of anoxia. Similarly, GSK-3 β inhibitors significantly reduced ATP consumption when sodium cyanide was used to stop mitochondrial respiration and also when the mitochondria were deenergized by uncoupler addition. This reduction in ATP consumption could be due to inhibition of ATP entry into the mitochondria through VDAC and/or ANT or to inhibition of the F₁F₀ ATPase. To clarify the site of the inhibitory effect on ATP consumption, we measured AMP production from exogenous ADP, which requires transport across the outer mitochondrial membrane but not transport across the inner membrane or metabolism in the matrix, and we found that GSK-3 β inhibitors slow AMP production similar to their effect on ATP consumption. This suggests that GSK-3 β inhibitors are acting on outer mitochondrial membrane transport. Since GSK-3 β is a kinase, we wanted to determine if protein phosphorylation might be involved in regulating adenine nucleotide transport. To identify proteins that might be involved in the cardioprotective effect of GSK-3 β inhibitors, we performed western blot and 1D/2D gel phosphorylation site analysis using phos-tag staining, and we identified proteins that had decreased phosphorylation in hearts treated with GSK-3 β inhibitors. LC/MS analysis revealed one of these proteins to be VDAC. Taken together, we found that GSK-3 β mediated signaling may modulate the outer membrane of the mitochondria, which is involved in the regulation of ATP consumption in anoxic mitochondria. Both proteomics and adenine nucleotide transport data suggest the involvement of VDAC in GSK-3 β inhibited cardioprotection and suggest that VDAC has an important regulatory role in ischemia-reperfusion injury.