Loss of insulin signaling and the onset of mitochondrial dysfunction and lipotoxic cardiomyopathy in the diabetic heart

Background

Insulin signaling plays a key role in substrate metabolism in the heart. Activation of insulin receptors on cardiomyocytes initiates phosphatidylnositol 3-kinase (PI3K) and Akt signaling pathways. These signaling pathways regulate gene expression, growth, and metabolism. Insulin resistance, type 2 diabetes, and obesity are associated with cardiac dysfunction. A recently recognized pathogenic mechanism is accumulation of triacylglycerol (TG) in cardiac muscle termed "lipotoxic cardiomyopathy" (3). Cardiomyocyte insulin receptor knockout (CIRKO) mice have been used to isolate the various effects of insulin signaling on substrate metabolism in cardiomyocytes (1). Of particular interest are the effects that lack of insulin signaling has on the mitochondria in the heart cells of these genetically engineered mice. Observations indicate that as CIRKO mice age, lack of insulin signaling in the heart leads to increased mitochondrial dysfunction as evidenced by inability of mitochondria to oxidize glucose and fatty acids (2). The basis for mitochondrial dysfunction appears to be an initial switch in substrate utilization that increases mitochondrial free fatty acid (FFA) flux, and decreases glucose utilization. Increased FFA flux produces reactive oxygen species (ROS). Increased production of ROS precipitates oxidative injury and uncouples respiration from oxidative phosphorylation. This in turn limits mitochondrial ATP synthesis. Knowing that cardiac performance is reduced in diabetic hearts (4), we believe decreased mitochondrial function could be an important mechanism that leads to lipotoxic cardiomyopathy and heart failure in diabetics.

Hypothesis

Accumulation of TG is a recognizable feature of a diabetic heart. Whether or not impaired mitochondrial insulin signaling predisposes hearts to lipotoxic injury is unknown. We hypothesize that lack of insulin signaling, and the resulting impairment in mitochondrial substrate metabolism will contribute to and accelerate the development of lipotoxic cardiomyopathy in diabetes.

Methods

To test our hypothesis that mitochondrial dysfunction is a key mechanism in myocardial TG accumulation, we will use CIRKO and wild type mice. There will be two groups of CIRKO mice tested, one group will be injected intraperitoneally with 50 mg/kg of streptozotocin (a diabetes inducing factor) for 5 consecutive days and the other group will not. Similarly with the wild type mice, one group will be injected with streptozotocin and the other group will not. This streptozotocin protocol induces diabetes in the majority of mice injected within 10 days. Blood glucose levels will be measured on alternate days from the tail vein of the mice using a glucose meter to document the severity of diabetes. Plasma FFAs will also be measured to confirm that diabetes leads to elevated systemic fatty acid concentrations. After these initial validation measurements, the mice will be sacrificed in two groups, one at approximately two weeks, and the other at four weeks. Hearts will be analyzed as follows: (1) Mitochondrial substrate metabolism will be measured using saponin-permeabilized fibers. (2) Long chain fatty acid acyl-CoA content will be determined using an assay, which detects the presence of NADH in a reaction catalyzed by the enzyme α ketoglutarate dehydrogenase. (3) Histological sections will be made and stained for accumulation of lipid. If our hypothesis is correct, we will expect to see an accelerated rate of mitochondrial dysfunction in streptozotocin treated CIRKO mice versus streptozotocin treated wild type mice and non-diabetic CIRKO, and thus will be associated with increased accumulation of long chain fatty acid and increased intra-myocardial lipid.

Data Analysis

Quantitative differences between the four groups will be analyzed statistically using analysis of variance. A $p<0.05$ will be accepted as indicating a significant difference.

How our study will improve upon previously published work in this field

This study will help to shed light on a possible mechanism by which impaired insulin signaling will lead to lipid accumulation in the hearts of diabetics. Specifically, this study will determine (1) If increased FFA flux will accelerate mitochondrial dysfunction in the insulin resistant heart and (2) If insulin resistance will accelerate the development of a lipotoxic cardiomyopathy. Dr. Boudina's efforts have already made tremendous strides in elucidating the mechanism by which insulin resistance leads to mitochondrial malfunction. This study will enhance our understanding of the link between diabetes and cardiovascular disease.

What I hope to learn from this summer experience

I am excited at the opportunity to work with Dr. Boudina. I consider it a privilege to be part of an ongoing project which has already yielded a number of abstracts and publications. It has already been a tremendous experience to propose a hypothesis for an experiment. I will not only be able to collect and analyze data in the given time frame, but the project will add knowledge to an area of medicine which to me is intriguing.

Cardiology and endocrinology are medical professions that interest me and in many ways go hand in hand. Gaining exposure to scientific research will help me to be an enlightened clinician who understands the molecular basis for disease. The knowledge I am gaining during these first two years in medical school will be enhanced by this summer opportunity. Proposing a hypothesis, conducting an experiment to test that hypothesis, collecting data from that experiment, analyzing the data collected, and writing the results will be a valuable research experience.

References

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