Introduction

Chronic hyperglycemia is a major condition of diabetes and it leads to vascular complications in diabetic patients. In non-diabetic patients, acute hyperglycemia also results in decreased wound healing and immune function, increased myocardial infarction size post myocardial infarction, and mortality. These effects of hyperglycemia are initiated by vascular endothelial dysfunction which is characterized by increased levels of reactive oxygen species (ROS) and decreased levels of endothelial-derived nitric oxide (NO). Endothelial NO synthase (eNOS) produces NO and is responsible for maintaining an anti-inflammatory surface to facilitate blood flow. NADPH oxidases are a principle source of superoxide (SO) during inflammation [1]. SO can directly quench NO by forming peroxynitrite (ONOO-), which induces eNOS uncoupling and additional ROS release (see figure 1). Facilitate blood flow. NADPH oxidases are a principle source of superoxide (SO) during inflammation [1]. SO can directly quench NO by forming peroxynitrite (ONOO-), which induces eNOS uncoupling and additional ROS release (see figure 1).

Hypothesis

We hypothesized that acute hyperglycemia (200 mg/dL) would increase blood H2O2 levels by 3 ± 0.42 μM with 5μM ML171 (*p<0.05, **p<0.01 vs Glucose). Hyperglycemia significantly increased blood H2O2 levels by 3 ± 0.42 μM (p<0.05). ML171 (1 and 5 μM) attenuated the hyperglycemia-induced increase in blood NO levels by 101.41±7.86 nM (P<0.01, n=5) and 85.95±8.13 μM (P<0.01, n=5) respectively at 180 min. Saline showed no change throughout the experiment.

Methods

Male Sprague-Dawley rats (257-325g; Charles River, Springfield, MA) were anesthetized and subsequently infused with saline, 30% glucose, or 30% glucose with 20 µg/Kg or 100 µg/Kg ML171 (approximately 1 or 5 µM in blood) via the cannulation of jugular vein. Hyperglycemic conditions (200 mg/dL) were induced, maintained, and monitored as previously described [4]. Mean arterial blood pressure (MABP) was monitored through catheterization of the carotid artery. Blood NO and H2O2 levels were measured in real time by NO or H2O2 microsensors as previously described (see figure 3) [4]. All data in the figures are presented as means ± S.E.M. The data were analyzed by ANOVA using the Student-Newman-Keuls post hoc test, p<0.05 were considered to be statistically significant.

Results

Figure 3. Nitric oxide and hydrogen peroxide sensors in the femoral artery.

Figure 4. Blood glucose changes throughout 180 minute experiment with 30% D-glucose. Blood glucose levels were maintained at ~200 mg/dL in all study groups.

Figure 5. Mean Arterial Blood Pressure among groups taken every 20 minutes for 180 minute experiment. MABP was maintained between 100-120 mmHg in all study groups.

Figure 6. Comparison of blood NO levels relative to saline among 30% D-glucose, 30% D-glucose with 1μM ML171, and 30% D-glucose with 5μM ML171 (**p<0.01 vs Glucose). Hyperglycemia significantly increased blood NO levels by 101.41±7.86 nM (p<0.01). ML171 (1 and 5 μM) significantly decreased blood NO levels by 1.86±0.89 μM (p<0.05) and 4.85±1.02 μM (p<0.05) respectively at 180 min. Saline showed no change throughout the experiment.

Figure 7. Comparisons of blood H2O2 levels relative to saline among 30% D-glucose, 30% D-glucose with 1μM ML171, and 30% D-glucose with 5μM ML171 (**p<0.01 vs Glucose). Hyperglycemia significantly increased blood H2O2 levels by 3 ± 0.42 μM (p<0.05). ML171 (1 and 5 μM) significantly decreased blood H2O2 levels by 1.86±0.89 μM (p<0.05) and 4.85±1.02 μM (p<0.05) respectively at 180 min. Saline showed no change throughout the experiment.

Conclusions

Our results indicate that NOX1 activation is a key mechanism contributing to acute hyperglycemia-induced oxidative stress and NO reduction in vascular tissue. Furthermore, inhibition of NOX1 may mitigate the deleterious effects of acute hyperglycemia. The outcomes from this study suggest that ML171 may be a therapeutic tool to attenuate vascular dysfunction associated with diabetic patients.

References