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Project Summary/Abstract
Impairment in insulin-stimulated glucose uptake is a major risk factor for developing Type 2 Diabetes. Insulin promotes glucose uptake through translocation of the facilitative glucose transporter 4 (GLUT4). GLUT4 is localized to the main insulin-responsive tissues, skeletal muscle and fat, but also to organs and cell types that do not require insulin for glucose uptake, such as CNS neurons. Deletion of insulin receptors in GLUT4-expressing tissues of mice (GIRKO) causes insulin-resistant diabetes and substantial insulin resistance in liver. This project studies whether the same mechanisms allowing GLUT4 translocation from the cytoplasm to the plasma membrane in the adipose tissue and muscle contribute to glucose uptake in the brain. Single cell microscopy and a fluorescent glucose analog will be used to visualize the uptake of glucose at the plasma membrane location where GLUT4 is expressed and determine whether glucose uptake occurs in a specific region of the neuron (e.g. synapses vs. soma). These experiments will be performed in primary cultures of neurons from the mouse hypothalamus. A high-resolution microscopy technique, total internal reflection microscopy (TIRF), will visualize a virus containing labeled GLUT4 in primary neuronal culture. Quantitation of labeled GLUT4 at the plasma membrane can be performed before and after insulin stimulation to determine the insulin responsiveness of these GLUT4 expressing cells. In the second part of the proposal, loss- or gain-of-function of hypothalamic insulin action mouse models will be generated by deleting the insulin receptor or Foxo1, respectively, in cells that express Nkx2.1. These models are expected to aid in the study of hypothalamic insulin action and its effects on insulin secretion from the pancreas and hepatic glucose production. GLUT4 localization in the primary neurons from these loss- and gain-of-function models will also be determined. The goals of these studies are to determine whether neuronal GLUT4 translocates to the plasma membrane and under what conditions, and how the loss or gain of insulin sensitivity in the hypothalamus affects the function of GLUT4-expressing neurons and the resulting effects on the periphery.