population level, we used genetically-encoded fluorescent indicators selectively expressed in alpha cells. Imaging intact mouse islets with these indicators in 3D responding to treatments in real time yields hundreds of individual alpha cell recordings per experiment. Calcium imaging showed reproducible heterogeneous responses to a panel of known physiological potentiators of glucagon secretion such as arginine vasopressin, epinephrine, and amino acids. Separate dose response experiments revealed that the proportion of alpha cells responding to each signal plateaus at different proportions of alpha cells. The calcium data correlate both with direct glucagon secretion levels as well as cAMP measurement. Our findings highlight previously unappreciated levels of functional heterogeneity among alpha cells and demonstrate that alpha cells are not a single uniform unit. Our observations suggest that dose-dependent increases in glucagon secretion in response to different physiological cues may be the result of mobilizing progressively larger proportions of the total alpha cell mass. We hypothesize that this functional heterogeneity is a built-in mechanism through which different physiological cues elicit graded glucagon responses from the alpha cells.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

Hyperglycemia-Induced Metabolic Reprogramming Mediates a Proatherogenic Phenotype in Healthy Human Monocytes

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Introduction: Poor glycemic control is considered an important contributor to cardiovascular disease in patients with diabetes. Episodic hyperglycemia as a surrogate for glycemic variability promotes monocyte adhesion and increases the prevalence of proinflammatory monocytes within atherosclerotic plaques of patients with diabetes. We previously found that acute hyperglycemia-induced a pro-inflammatory phenotype and promoted the development of foamy monocytes by increasing total cholesterol deposition, cholesterol ester, and free cholesterol content by enhancing oxidized LDL uptake. However, the mechanism by which acute hyperglycemia induces monocyte cholesterol deposition and inflammation remains unknown. **Methods:** Monocytes isolated from healthy individuals (age range 20–40; n=5) were cultured in low (5mM) or high (16.7mM) glucose conditions with or without a glycolysis inhibitor (2-deoxyglucose, 2DG, 5 mM) or an endoplasmic reticulum stress inhibitor (4-phenylbutyric acid, PBA; 20mM) for 6 hrs. After treatment, cytokine release, oxidized LDL uptake, and metabolic assays using Seahorse Technology were performed. **Results:** Healthy human monocytes exposed under high glucose conditions showed a pro-atherosclerotic phenotype with higher levels of the pro-inflammatory cytokines, TNFα(median of differences 6.34 pg/ml, p=0.002) and IL1β(12.04 pg/ml, p=0.003), and increased oxidized LDL uptake (5062ug Dil-Ox LDL/mg, p=0.001). Furthermore, hyperglycemia resulted in higher levels of glycolysis (basal glycolysis 12.94 pmol/min, p=0.01; basal proton efflux rate 15.5 pmol/min, p=0.03) and mitochondrial respiration (percentage of respiratory capacity 16pmol/min p=0.04), suggesting a significant alteration in the metabolic programming of these monocytes. Treatment with 2-DG or PBA attenuated the pro-atherosclerotic phenotype induced by hyperglycemia, promoting a reduction of cytokine release, a reduction of oxidized LDL uptake, and near normalization of the glycolic rate and mitochondrial respiration, stabilizing cellular bioenergetics. **Conclusions:** Altogether, our results suggest that monocyte ER stress in response to acute hyperglycemia promotes a hypermetabolic state characterized by a proinflammatory and proatherogenic monocyte phenotype. Therefore, acute hyperglycemia is a potential mechanism promoting atherosclerosis in patients with type 2 diabetes.

Diabetes Mellitus and Glucose Metabolism

BENCH TO BEDSIDE: NOVEL MECHANISMS IN DIABETES AND METABOLISM

LGR4 and Its Extracellular Domain as Novel Regulators of ß-Cell Survival and Proliferation

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Our lab has shown that RANK (Receptor activator of the NF-κB) by interacting with its ligand, RANKL, inhibits ß-cell proliferation and survival; which can be reversed by Osteoprotegerin (OPG). Recently, the G protein-coupled receptor LGR4 (leucine-rich repeat-containing G protein-coupled receptor 4), which binds R-spondin (RSPO), was identified as a novel receptor for RANKL in osteoclast precursor cells. Thus, RANKL can bind two distinct receptors, RANK and LGR4 in osteoclasts, leading to opposite effects on osteoclastogenesis. LGR4 is expressed in rodent and human ß-cells, but the role of this receptor in ß-cells remains unknown. We postulated that LGR4 through its interaction with RANKL is involved in regulating ß-cell survival and proliferation. Our data indicate expression of specific LGR4 family members, Lgr4, Rank, Rankl, is modulated by stressors, such as cytokines, ER stress, diabetes and aging, in INS1 cells, rodent and human islets. Knocking down Lgr4 in INS1 cells or rodent islets has no significant effect on ß-cell proliferation but is detrimental for ß-cell survival in basal and cytokine-stimulated conditions. We also propose that the soluble extracellular domain of LGR4 (LGR4-ECD), which binds to its ligands (RSPO/RANKL), holds therapeutic potential like OPG, by inhibiting the interaction between RANKL/RANK. At 200ng/ml LGR4-ECD significantly enhances young adult (8-12-week-old) and aged (1.y.o.) rodent ß-cell proliferation, as well as human ß-cell proliferation, in islets from not only control subjects (45±17 y.o.), but also with Type 2 diabetes (48±7 y.o.). Additionally, LGR4-ECD significantly promotes