

Measuring Islet Sensitivity to Glucose Stimulated Insulin Secretion in the LEW.1WR1 Rat

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Introduction

The onset of type 1 diabetes (T1D) is caused by a shortage of insulin and insulin-producing cells because of an autoimmune or toxicant-induced cell death. The LEW.1WR1 (1WR1) rat is predisposed to developing T1D due to a combination of genetic and an immune stimulation trigger. The 1WR1 rat has a mutation in its FAT10 gene, which is an ubiquitin-like protein and was identified as a T1D susceptibility gene by the Diabetes Autoimmunity Study in the Young (2). This mutation causes the 1WR1 rat to overexpress FAT10 and exhibit high fasting blood insulin levels. It is unclear why their blood insulin levels are increased, if this plays a role in T1D susceptibility and if the over responsive beta cells (insulin producing cells contained within the pancreas) are the cause. Our hypothesis is that specific beta cell changes in the 1WR1 rat that increase their susceptibility to T1D. We hope to observe if isolated islets of Langerhans (cluster of cells that contain beta cells) from 1WR1 (T1D susceptible) rats respond differently to varying glucose concentrations than LEW.ssNHsd (ssNHsd) rats (T1D resistant).

Methods

Animals

LEW.ssNHsd and LEW.1WR1 rats were obtained from Envigo (Indianapolis, IN) and Biomere (Worcester, MA) respectively. SsNHsd and 1WR1 were approximately 4 weeks old at time of harvest of islets. Protocols were approved by UAH IACUC.

Islet Isolation

Rats were anesthetized and exsanguinated. The bile duct was cannulated and the pancreas was inflated with Clzyme (Indianapolis, IN). After digestion, islets were gradient purified and hand-picked. Cells were cultured overnight in RPMI 1640 (Gibco; Waltham, MA) supplemented with 1% pen-strep (Gibco; Waltham, MA), and 5% exosome free Fetal Bovine Serum (Gibco; Waltham, MA) media in a 5% CO₂ incubator at 37 °C overnight.

Insulin Secretion Assay

30 islets were placed in triplicate samples in microcentrifuge tubes. Islets were equilibrated in low glucose solution of 2mM-glucose Krebs Ringer Buffer (KRB) for 30 minutes. Cells were then incubated in differing concentrations of 2mM, 10mM, and 20mM-glucose KRB solutions for 1 hour at 37 °C (5% CO₂). Insulin secreted (1:20 dilution) and insulin content (1:400 dilution) were assayed using a Thermo Scientific Rat Insulin ELISA Kit according to the manufacturer's instruction.

Results and Discussion

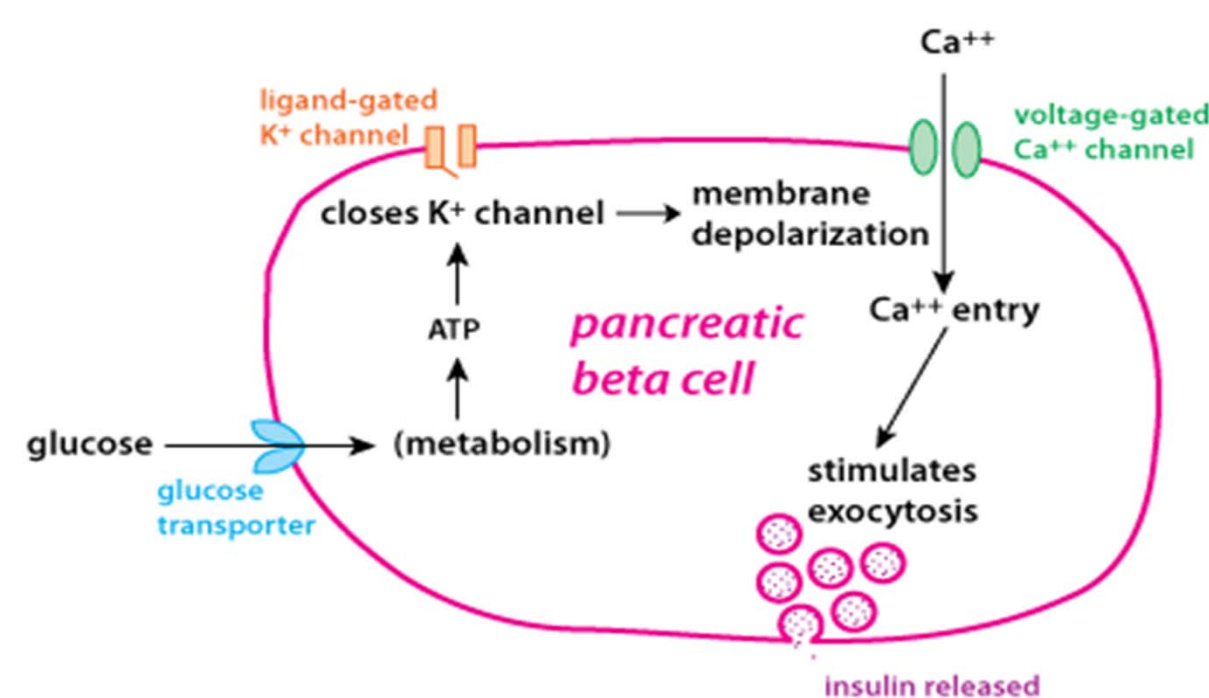


Figure 1. Glucose-stimulated insulin secretion This figure illustrates the process of glucose-stimulated insulin secretion. Glucose is taken up and metabolized to produce ATP (energy). ATP signals the ligand-gated potassium channel to close which causes membrane depolarization. Depolarization signals the voltage-gated calcium channels to open which stimulate the exocytosis of insulin granules. Figure 1 from (1).

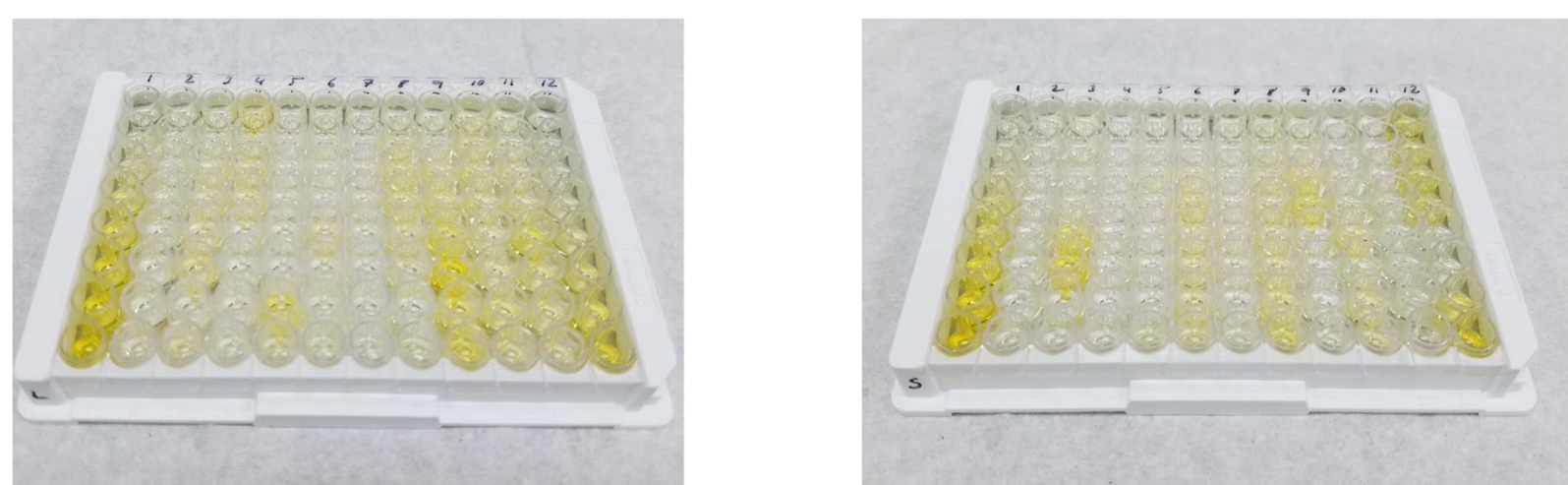


Figure 2. Enzyme-Linked ImmunoSorbent Assay (ELISA) ELISAs are plate-based assays used to detect substances like hormones, proteins, and peptides. The pictures of the ELISAs are above (1WR1 above left and ssNHsd above right). These were run to measure insulin concentration of our samples. The darker colored wells have a higher concentration samples while the lighter wells have a lower concentration samples. The first column on the far left side is a set of standards with known concentrations that allow for the quantitation of insulin.

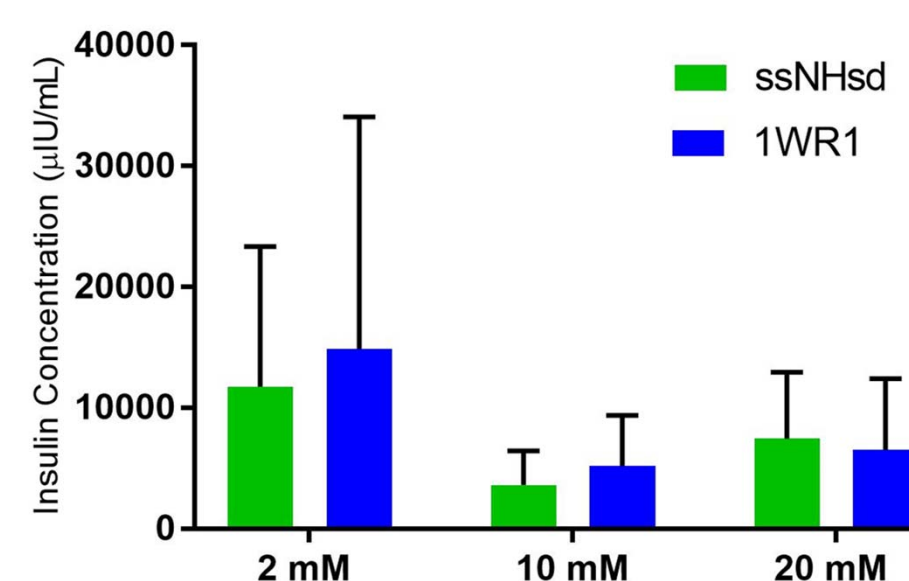


Figure 3. Insulin Content of Islets We were able to calculate the concentration of insulin content in the islets of both strains of rat using the data from the ELISA. From the graph however, there are no apparent differences in the amount of Insulin in the islets. SD were calculated from n=6 samples.

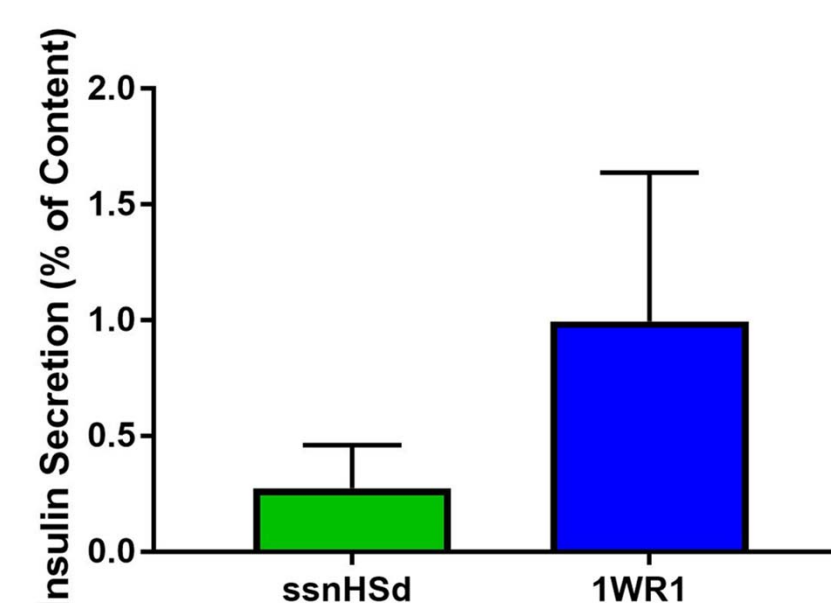


Figure 4. Insulin Secretion of islets at 2 mM glucose We measured the concentration of insulin secreted by the islets and normalized this to total insulin content in the islet. The ssNHsd rat seems to secrete less insulin than the 1WR1 rat, however, these values were not significantly different. Unfortunately, we were unable to compare the 10 and 20 mM glucose experimental values to the 1WR1 rat because the insulin levels in the ssNHsd rat were outside of the range of the standard curve. SD were calculated from n= 3 ssNHsd and n=7 1WR1 samples.

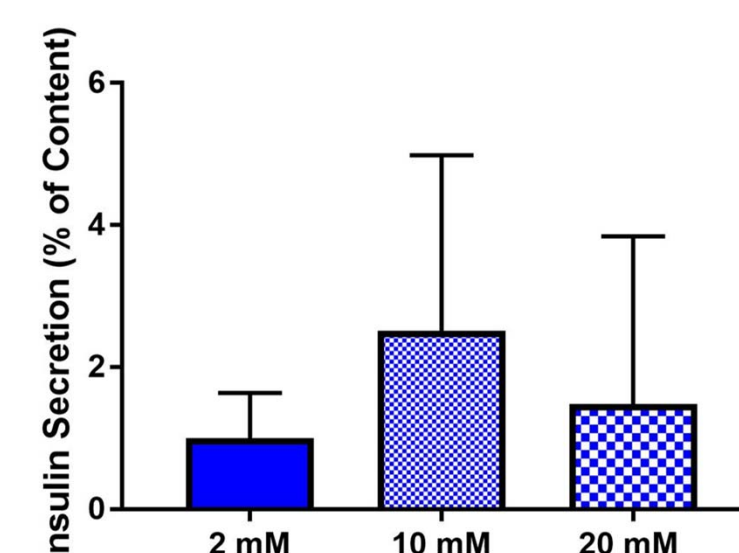


Figure 5. Insulin Secretion of LEW.1WR1 islets This graph depicts insulin secretion as a percent of the content as a means of normalizing concentration. The islets appear to increase insulin concentrations as glucose concentration increases. This shows that the islets have a response to glucose, but due to the lack of control data, it is unclear if there is a difference in glucose-stimulated insulin sensitivity. SD were calculated from n=7, n=5, and n=4 samples.

Conclusion

It appears that the 1WR1 islet may be more responsive to Glucose-stimulated insulin secretion, but we cannot say this with certainty without more data points. The insulin concentration for several of the ssNHsd rats was not within the standard curve of the assay. This suggests two things: the lack of a response to the glucose could be due to the health of the islets, or the samples were too diluted to be measurable. In order to identify the cause, we will have to perform more tests. We did learn how to optimize the conditions of a ELISA for future studies in the 1WR1 rat from this experiment.

References

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