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Ultrasound activated TRPV1 remote regulation of insulin release

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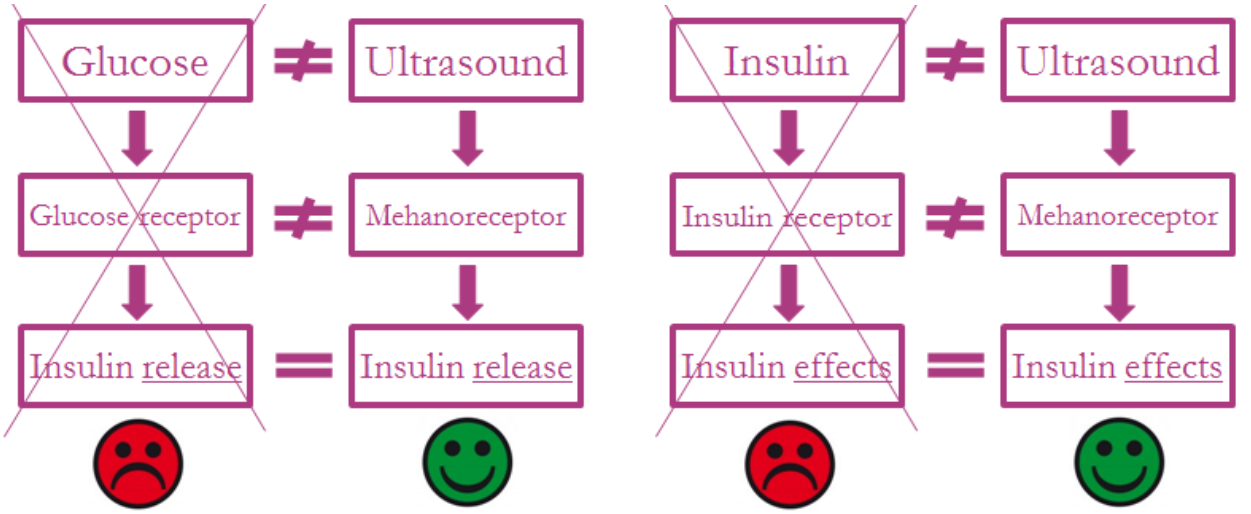
The main idea is to speed up the process which requires glucose metabolism and which is synthesis regulated into the process which is ultrasound regulated (on the phosphorylation level).



Part 1: Ultrasound as stimuli for insulin release

The project proposal is based on the following premises:

1. Human islets (β cells in pancreas) express several members of the transient receptor potential (TRP) family of ion channels the role of which is currently unknown.
2. The objective of β-cell electrical activity is to produce the $[Ca^{2+}]i$ elevation that triggers exocytosis of insulin granules.
3. TRPV1 mechanoreceptor studies show $[Ca^{2+}]i$ elevation in the presence of the stimuli.
4. Studies on mouse islets have established that the molecular machinery involved in insulin exocytosis is similar to that controlling neurotransmitter release.

In the living organisms, mitochondrial oxidative metabolism is essential for glucose-induced insulin secretion in pancreatic islets. My suggestion is to speed up and transform that signaling pathway into ultrasound-induced insulin secretion by using the methods of synthetic biology. The foundation of the idea lies on the resemblance of the two pathways – on one side TRPV1 caused Ca^{2+} conductance and on the other side glucose caused ATP/ADP changed ratio which again causes Ca^{2+} conductance. Therefore, these two pathways might result in the same outcome – increase of the intracellular Ca^{2+} concentration, which would in turn cause the exocytosis of insulin granules. Interestingly, the stimulus is completely different and provides a potential therapeutic benefit for nearly 5% of the world population who have diabetes mellitus, especially T2DM.

Part 2: Effects of insulin in the absence of insulin

The project proposal is based on the following premises:

1. Insulin receptor is a tyrosine kinase which auto-phosphorylates when exposed to the stimuli.
2. Dimerization of the insulin receptor (after alternate splicing) in necessary for the phosphorylation to take place.
3. Small molecule gated designed tyrosine phosphatases and orthogonal kinases have already been described.

In the absence of insulin, as it happens in T1DM, the effects of insulin obviously cannot occur. It knows to be very intimidating for those patients to inject insulin even though it is minimally invasive. However, it would represent an incredible advancement even in the field of endocrinology if the ability to regulate the insulin effects in absence of it was demonstrated and, additionally, doing it completely non-invasively. GLUT transporters could be placed under appropriate promotor to ensure the continuous expression (as the house-keeping gene). These GLUTs could be additionally modified to include the sequence for their accumulation in the ER, which would be released after the trigger signal. In would be optimal if one could design the dimer of tyrosine kinases which would be inactive unless simulated by the TRP or calmodulin. Furthermore, it would be even better if the insulin cascade could occur with fewer steps - ultrasound mediated release of the GLUT transporters – which would be possible either by:

a) Ca^{2+} mediated release of exocytosis vesicles including GLUT (by vesicle membrane proteins) or
b) Coupling TRP with the appropriate kinase which would need to (de)phosphorylate the GLUTs to activate them (instead of transport dependent (de)activation)

To obtain all of this, it would be necessary for the cells not expressing TRP to modify them with the means of synthetic biology (for example with CRISPR Cas9 or plasmid transfection).

Project implementation

Cell lines: TOP10 Chemically Competent E. coli, HEK cells, human β cell islets.

Stages needed (in short):

1. Choosing the most appropriate TRP receptors (for example TRPV1), determining the ultrasound frequencies to be used in the experiment, adequate controls and knock-outs.
2. Obtaining sufficient amounts of various genes – TRP, GLUT, GLUT-GFP, …
   (Designing PCR primers, performing PCR, purification, DNA electrophoresis, restriction, ligation, transduction of plasmid to the TOP10 by bacteriophage or heat-shock, lysis, purification, electrophoresis, DNA sequencing…).
3. Human cell cultures
   (Transfection of DNA plasmids into HEK cells or CRISPR Cas9 DNA modifications, antibiotics and medium changing every two days, splitting into flasks, DMSO -80°C freezing …)
4. Experiment: Stimulation of the cells by ultrasound.
   (Detection of the level of expression of TRPV1 and its location (by using GFP flag, protein purification, gene expression analysis…), $[Ca^{2+}]i$ elevation (micro-conductance), level of expression of insulin, level of exocytosis caused by TRPV1 activation, insulin mediated effects (by measuring the concentration of glucose)…)
5. Continuous optimization of the method and regular problem solving.
6. Design of the phosphorylation (in)activated GLUTs and design of the dimer of tyrosine kinase activated in the absence of insulin (ultrasound as the logic gate).

Innovativeness and feasibility

I consider the project suggestion to be very innovative with considerably good feasibility. In the last 20 years enormous advancement has been made in the field of insulin and DM research. In addition, it has been researched a lot about potential agonists and antagonists of insulin release and its effects as well as the molecular mechanisms underlying. Even though it has been achieved the insulin expression under different stimuli – by using optogenetics, it has not been described the successful method for the insulin expression under sonogenetic stimuli. Moreover, modification of the existing signal cascade instead of only activating it in different way could be claimed to be another innovation. In my opinion, it seems to be an appropriate continuation considering all the research currently being conducted in the above mentioned fields.

Potential problems and benefits

There are many potential problems. Even though I have dozens of ideas in many directions (I am capable of dreaming big) and some of them appear after reading research articles, I have very limited knowledge in the practical sense to determine their feasibility.

The main potential problems include (if I assume no difficulties with ultrasound mediated TRP activation):

1. insufficient TRP mediated elevation of $[Ca^{2+}]i$ to trigger exocytosis of insulin granules
2. difficulties designing the inactive tyrosine kinase which would dimerize/activate in the presence of TRP stimuli and/or GLUTs
3. inappropriate expression of TRP in the modified cells normally not expressing them

On the other side, the main potential benefits could be:

1. sonogenetics as the logical continuation of optogenetics regulated expression
2. potential therapeutic value of non-insulin mediated non-invasive insulin effects
3. development of the dimer kinase which could be used as a quick logic gate in many different pathways and the activity of which is regulated on the (de)phosphorylation level instead of synthesis, post-transcriptional modification by splitting or by different small molecules

How and why this idea can influence the development of synthetic biology

Not only would it show how modification of the existing pathways might have interesting consequences but also how different stimuli could be employed for their activation. Additionally, it would show the ability of synthetic biology to develop in that direction, which has not been frequently used so far. Consequently, it would surely represent certain 'paradigm shift' in the way of our understanding of the cell processes.

Moreover, if shown to be indeed beneficial, optimal to control, it might represent a step for DM treatment, cancer treatment as well as employment of other approaches for remote regulation of various genes (for example proton therapy because of the specificity of the Bragg peak).