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From opto- to radio-genetics

A switch in the wavelength

Juliane Proft[1] and Norbert Weiss[2,*]

[1]Hotchkiss Brain Institute; Department of Clinical Neuroscience; Calgary, AB Canada; [2]Hotchkiss Brain Institute; Department of Physiology and Pharmacology; University of Calgary; Calgary, AB Canada

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*Correspondence to: Norbert Weiss; Email: norbert.weiss@yahoo.fr

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The use of ion channels to control defined events in defined cell types at defined times in the context of living tissue or whole organism represent one of the major advance of the last decade, and optogenetics (i.e the combination of genetic and optical methods) obviously played a key role in this achievement.[1] Although the existence of light-activated ion channels (i.e ospin channels) has been known since 1971,[2] it took about 35 y before the concept of an ion channel used for bioengineering control of cell or tissue activity becomes reality.[3] From that moment forward, rhodopsine channels[4,5] (i.e blue light-gated non-specific Na^+ channels that depolarize cells thus increasing cell excitability) or halorhodopsin channels[6] (i.e yellow light-gated Cl^- channels that hyperpolarize cells thus decreasing cell excitability) have been extensively used to turn neurons on and off in response to diverse colors of light, with an extremely high temporal precision (i.e milliseconds range). Although optogenetics has been originally established in neuroscience, it addresses now to non-neuronal systems, including cardiac, smooth and skeletal muscles, glial cells or even embryonic stem cells.[7-9] However, although light stimulation allows control of cell excitability with a high spatio-temporal specificity, light waves present the disadvantage to not penetrate deep tissue, and implanted devices are required for in vivo light stimulation. In contrast to visible light-waves, radio-waves (i.e longer wavelength and lower frequency) can penetrate deep tissues with minimal energy absorption.

In a recent study published in Science,[10] the authors have examined the possibility to use radio-wave instead of light-wave to control cell activity and protein production in vivo. The results of this study indicate that bioengineering of a mammalian TRPV1 channel (i.e a temperature-gated Ca^{2+} channel) for radio-wave activation, coupled to a Ca^{2+}-dependent insulin gene transcription, can be used to stimulate insulin production in vivo and regulate plasma glucose when expressed in mice.

Contrary to light-gated ion channels (such as channelrhodopsine, bacteriorhodopsin or halorhodopsin) that naturally exist in earlier life forms, radio-wave-gated ion channel has never been described so far. To carry out their studies, the authors first engineered the temperature-sensitive TRPV1 Ca^{2+} channel[11-14] with iron oxide nanoparticles (FeNPs). For that, they introduced a histidine tag (His × 6) within the extracellular linker between domains I and II of the TRPV1 channel ($TRPV1^{His}$), and coated iron nanoparticles with anti-histidine antibodies. In vitro radio-wave stimulation of iron nanoparticles (20 nm diameter) results in a significant increase in local temperature of around 15°C, expected to stimulate opening of TRPV1 and Ca^{2+} influx when coated to the channel. Incubation of HEK cells expressing $TRPV1^{His}$ channels with coated nanoparticles results in a specific accumulation of nanoparticles at the cell surface as proved by electronic microscopy. Using whole-cell Ca^{2+} fluorimetry on these cells, the authors demonstrated that radio-wave stimulation (465 KHz) effectively produce an intracellular Ca^{2+}

elevation that is not observed in control conditions with non-coated $TRPV1^{His}$, indicating that radio-wave stimulation specifically activates $TRPV1^{His}$-coated channels. It is worth to mention that introduction of histidine tag in TRPV1 channel does not alter temperature-dependent activation of the TRPV1 channel.

In order to functionally link the signal transduction of $TRPV1^{His}$ to the insulin production, the authors then engineered a cDNA encoding the human pro-insulin (with a furin cleavage sites to allow the conversion of pro-insulin into insulin in non β-pancreatic cells) under the control of Ca^{2+} response elements (serum response, cyclic adenosine monophosphate response and nuclear factor of activated T cell response elements). Radio-wave stimulation of HEK cells expressing $TRPV1^{His}$ nanoparticule-cotated and engineered human insulin gene results in a significant increase in insulin gene activation (as evidenced by an increase in insulin mRNA) and pro-insulin release with a maximal effect after about 45 min of stimulation. This effect is abolished after treatment of cells with 100 μM ruthenium red, a TRPV1 antagonist, indicating that radio-wave-dependent pro-insulin specifically depends on the activation of $TRPV1^{His}$ channels. In order to further investigate the potential of engineered radio-wave-gated TRPV1 channel in the control of insulin release in vivo, the authors developed xenografts from PC12 cells expressing $TRPV1^{His}$ channels in combination with the bioengineered human insulin gene. Whereas radio-wave stimulation of mice subcutaneously injected with the tumor had no effect neither on the insulin gene expression, nor on the blood glucose level, injection of coated nanoparticles into the tumor produced a specific radio-wave-dependent secretion of insulin associated with a significant decrease in blood glucose concentration, without any significant change in internal body temperature. However, although radio-wave-dependent production of insulin in vivo appears to efficiently modulate blood glucose concentration in wild-type animal, it would be interested to further evaluate this approach using diabetic animal models.

Finally, in order to improve the technique and avoid the injection of external nanoparticles, the authors modified and overexpressed the iron storage protein ferritin in HEK cells. Ferritin is able to form a naturally occurring paramagnetic iron nanoparticle.[15,16] It should also be mention that ferritin could be stably overexpressed in mice for over 2 y without any significant pathological side effects.[17] Expression of the fusion peptide composed of ferritin light chain, flexible linker region and ferritin heavy chain resulted in an increase in ferritin particles in the cells with some of them localized near the plasma membrane. Radio-wave stimulation of cells expressing ferritin, TRPV1 wild-type and the human insulin gene resulted in a significant increase in insulin gene activation and pro-insulin release, demonstrating that accumulation of ferritin might be sufficient to produce local temperature elevation under radio-wave stimulation and TRPV1 activation in cell culture. However, the number of functionally exposed TRPV1 channels to local temperature elevation produced by radio-wave stimulation of endogenous ferritin nanoparticles is certainly much less than those directly decorated with coated iron oxide nanoparticles, and although being efficient in cell culture, this approach requires further evaluation in vivo.

In summary, the authors have provided compelling evidences that bioengineering of temperature-gated ion channel for radio-wave activation represents an interesting approach to control cell activity and protein production in vivo. Control of blood glucose homeostasis in mice has been previously demonstrated using opto-genetic approaches.[18] Although visible light waves do not penetrate deep tissues, direct illumination remained efficient for activation of subcutaneous implants containing light-inducible transgenic cells. In the present study, the authors have shown that similarly to the opto-genetic approach, bioengineered radio-wave-inducible transgenic cells can be efficiently used to control insulin production and blood glucose concentration when injected subcutaneously in mice. However, one of the main advantages of radio-waves compare with light-waves relies in their capability to penetrate deep tissue, and they are therefore expected to activate radio-wave-inducible cells when injected not only subcutaneously but also more deeply in tissue. Further investigations will certainly determine the exact capability of radio-wave stimulation to control deep tissues.

Overall, bioengineering of temperature-gated ion channel for radio-wave activation definitively represents a novel and promising approach for external control of cell/tissue activity in vivo.

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