Gamma-aminobutyric acid (GABA) treatment blocks inflammatory pathways and promotes survival and proliferation of pancreatic beta cells

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Limitations of current therapies of diabetes

Do not prevent or reverse type 1 diabetes (no cure).

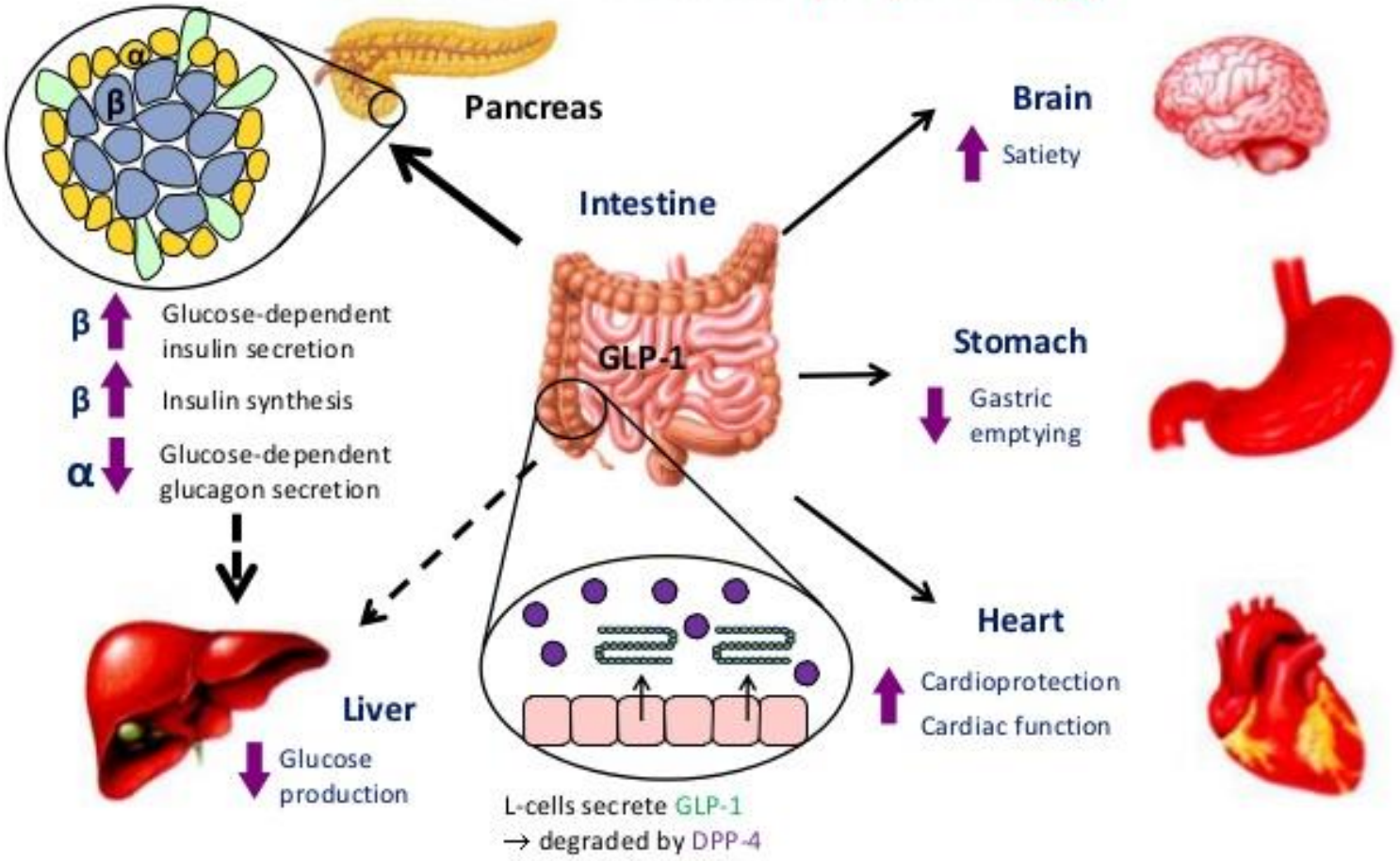
Minimal or no improvement in the survival of pancreatic beta cells (type 1 or 2).

Do not induce replacement or regeneration of beta cells (type 1 or 2).

EFFECTS NEEDED TO CURE TYPE 1 DIABETES

- Stop the autoimmune (inflammatory) reaction that kills beta cells.
- Increase the resistance of beta cells to injury.
- Stimulate the regeneration of beta cells, or replace these cells.
- Research aspect: human beta cells are different from mouse. Drugs must work on human cells.

GLP-1: an incretin hormone with multiple direct effects on human physiology



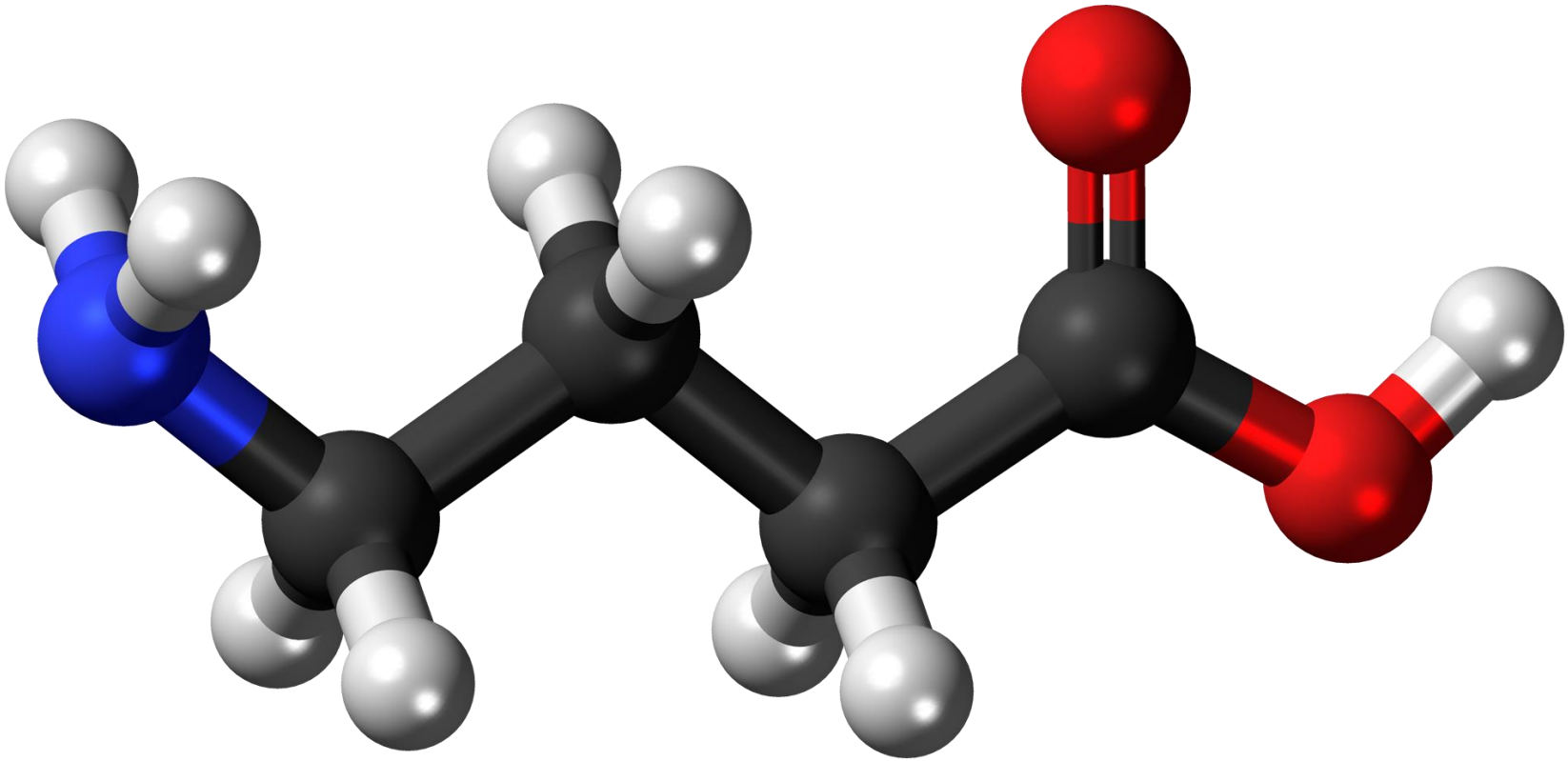
Adapted from Baggio & Drucker. *Gastroenterol* 2007;132:2131–57

GLP-1 receptor agonists

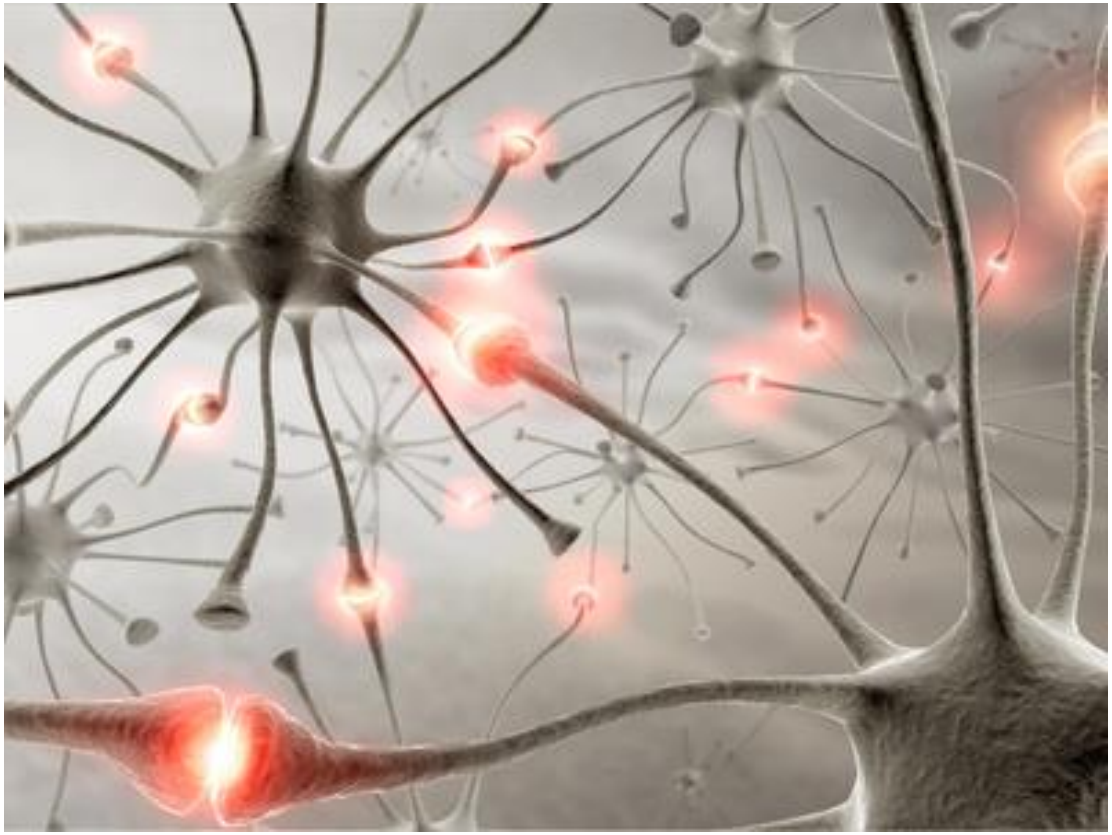
- Effective in the treatment of type 2 diabetes
- Several drugs available or under investigation: exenatide [Byetta], liraglutide, dulaglutide, etc.
- Not effective in type 1 diabetes (lack anti-inflammatory and regenerative capacity).

GABA

Gamma-aminobutyric acid



GABA is an inhibitory neurotransmitter in the brain, but also present in the pancreas



GABA

Brain:

Major inhibitory neurotransmitter.

Islets:

Inhibits α cells, but stimulates β cells.

Immune system:

Inhibits lymphocytes and macrophages

GABA RECEPTORS

Type A (GABA-A receptor):
Fast acting ligand-gated chloride channel (many variants).
Blocked by picrotoxin.

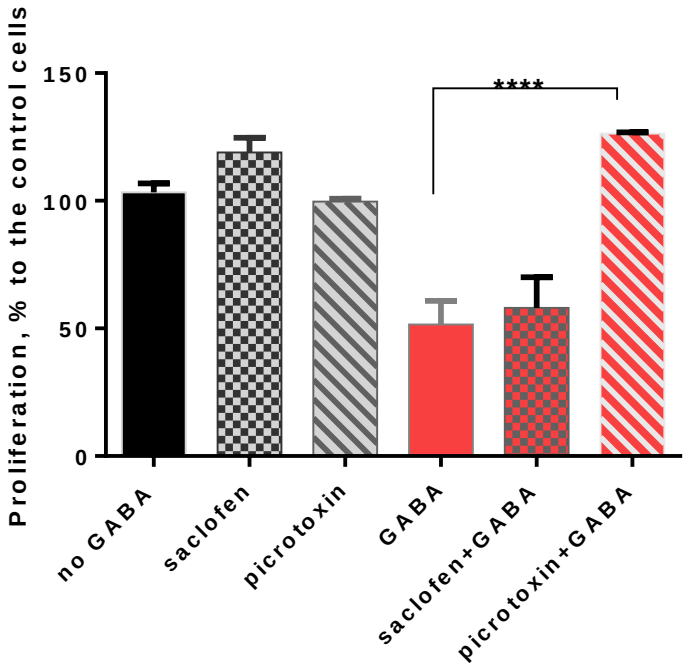
Type B (GABA-B receptor):
Slow acting G-protein coupled receptor.
Blocked by saclofen.

Neurons and islet cells: Express both receptors.

Lymphocytes: Type A only.

GABA suppresses proliferation of human T cells

(anti-CD3 antibody)



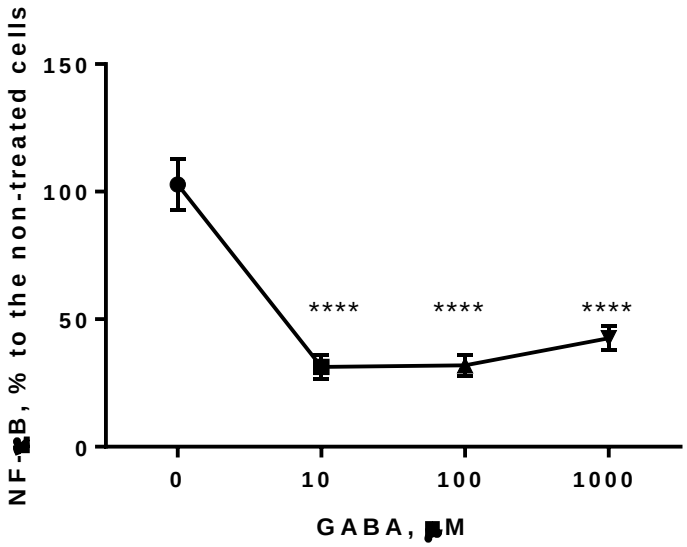
Immunotherapeutic effects of GABA in T1D

Strain/model	Immune effects	Disease course
NOD(prediabetic)	Th1 cells↓, IFN-γ↓ IL-12↓, Treg↑	prevented diabetes (insulitis↓)
NOD (diabetic)		reversed transiently
NOD-TCR8.3	CTL response↓	prevented (insulitis↓)
CD1 mice (low dose STZ, diabetic)	IL-1↓, TNF-α↓ IL-12↓, IFN-γ↓	reversed diabetes (insulitis↓, β-cell regeneration)

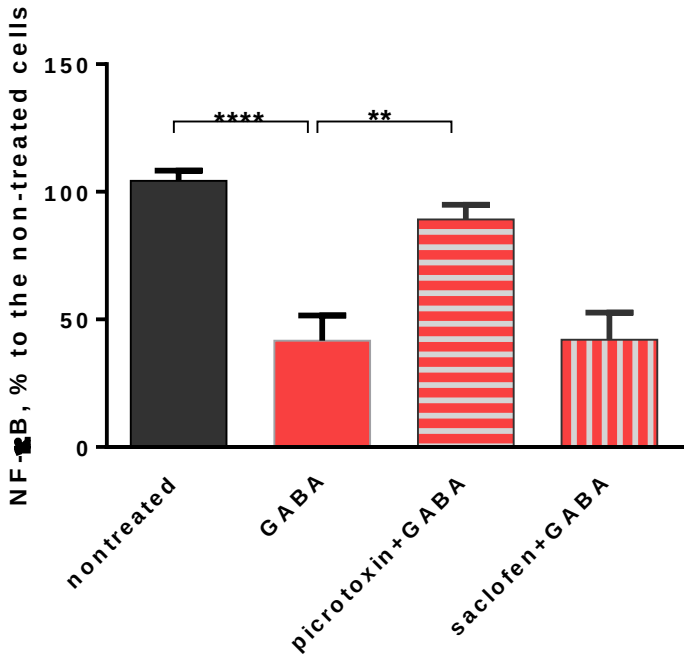
Reviewed in: Prud'homme et al. Autoimmunity reviews, 2015, 14:1048-56.

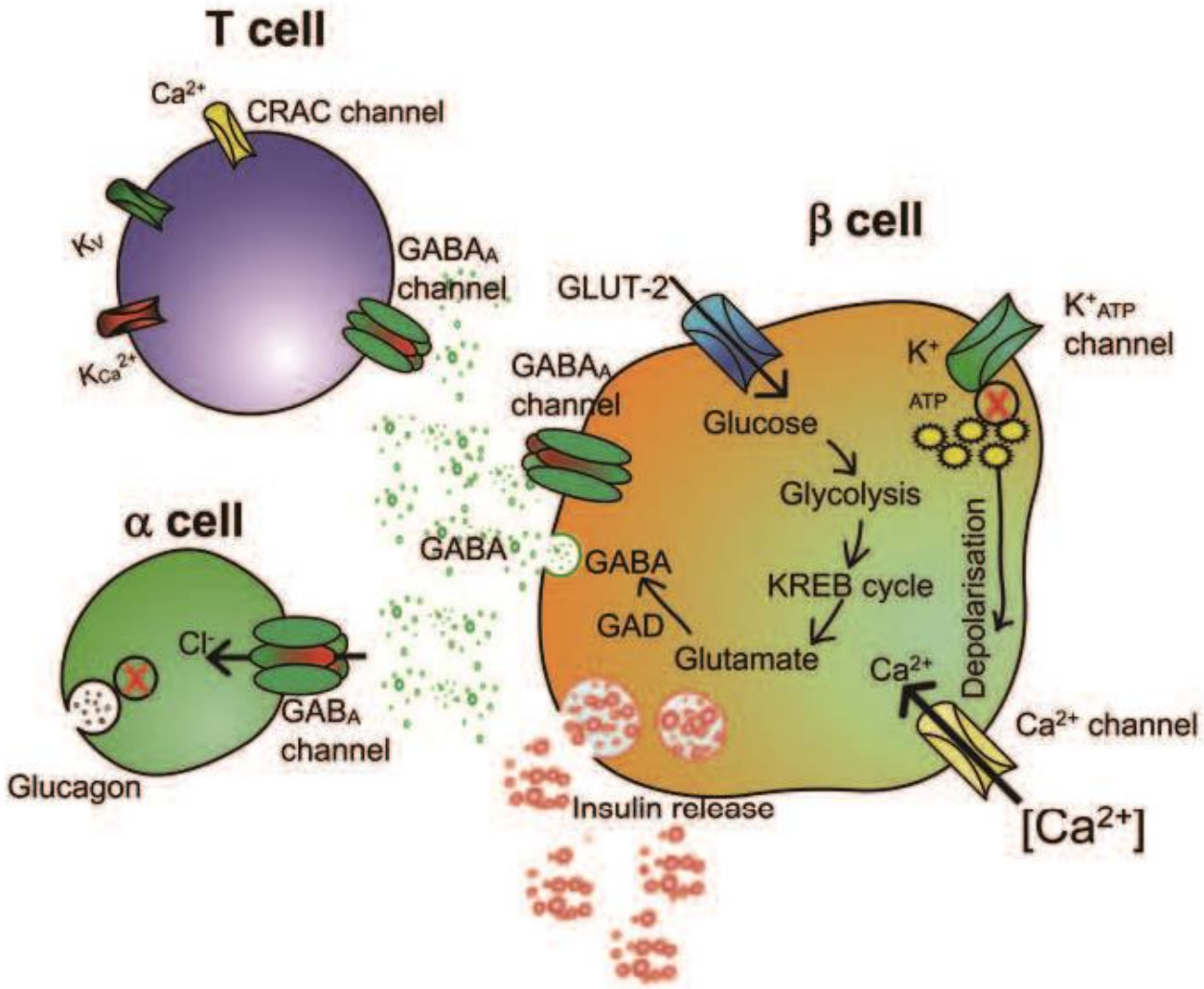
Inhibition of NF-kB in Mouse T cells

(anti-CD3/CD28 stimulated)



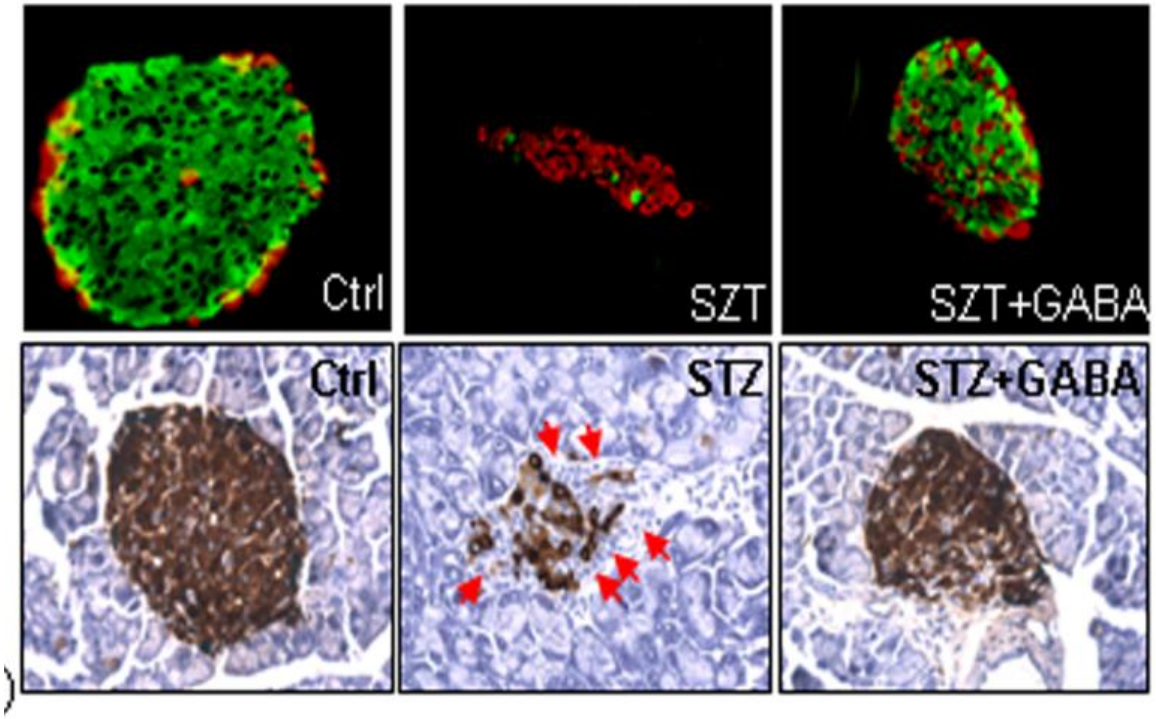
GABA suppresses activation of NF-kB in human islets through GABA-A receptors





GABA BLOCKS AUTOIMMUNE REACTION AGAINST BETA CELLS

GABA induces beta-cell regeneration



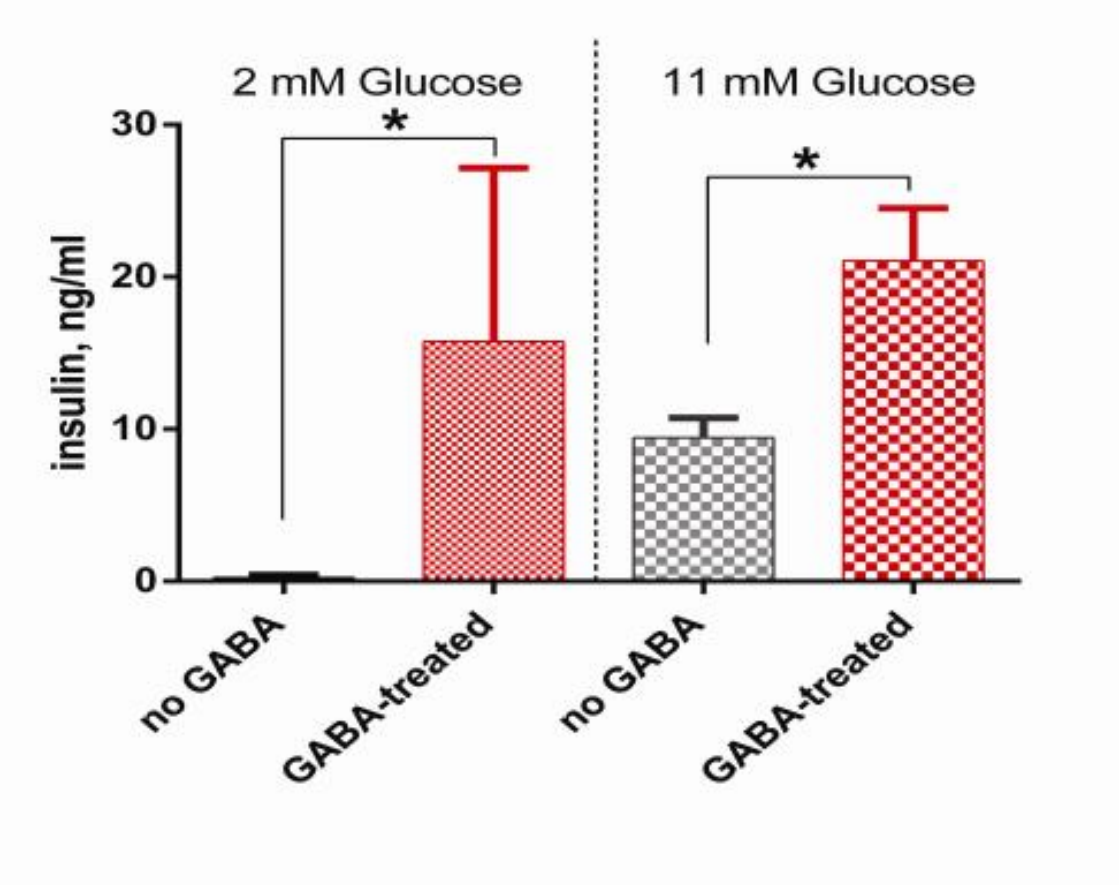
Mouse:

GABA prevents β-cell death, stimulates β-cell proliferation, increases insulin secretion, and promotes β-cell regeneration.

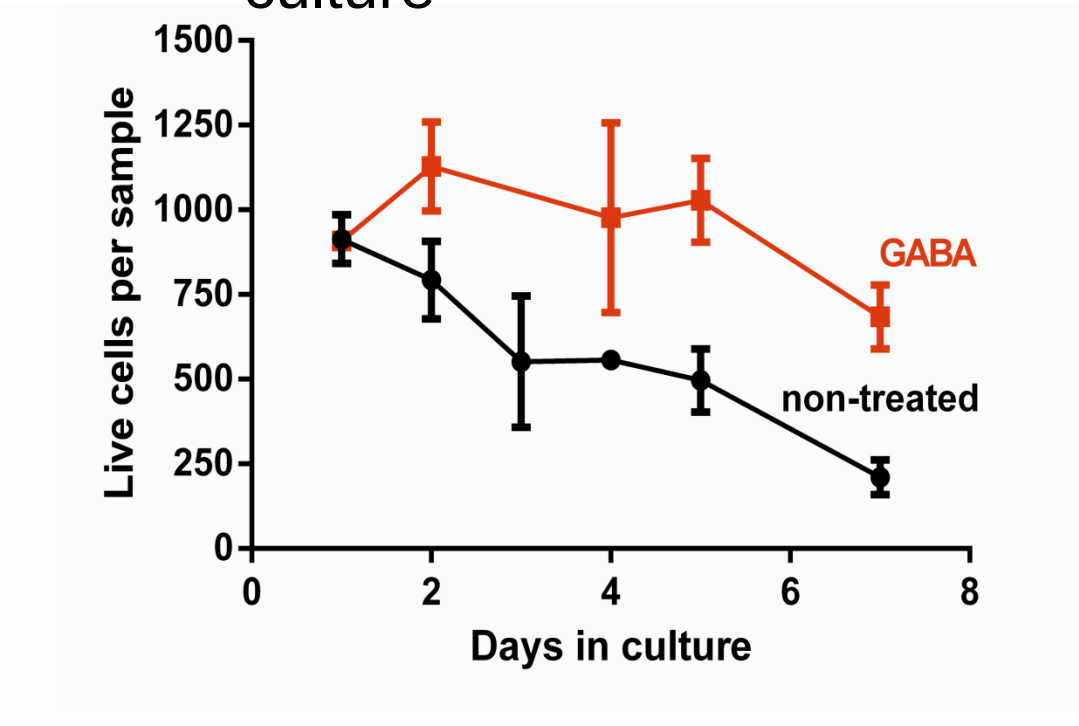
GABA exerts anti-inflammatory and immunosuppressive effects.

Humans: Similar to mouse??

GABA stimulates insulin secretion

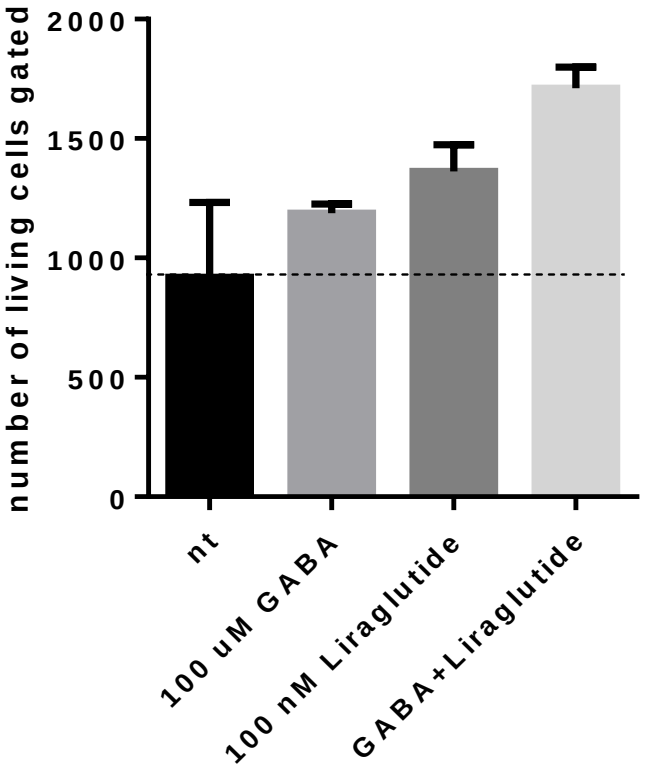


Human beta cells in culture



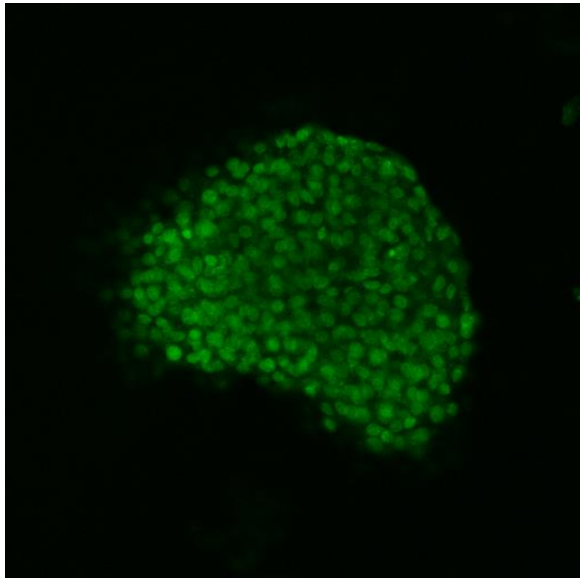
GABA and Liraglutide (GLP-1R agonist) ameliorate human beta-cell survival

(24 h in vitro; additive effect)

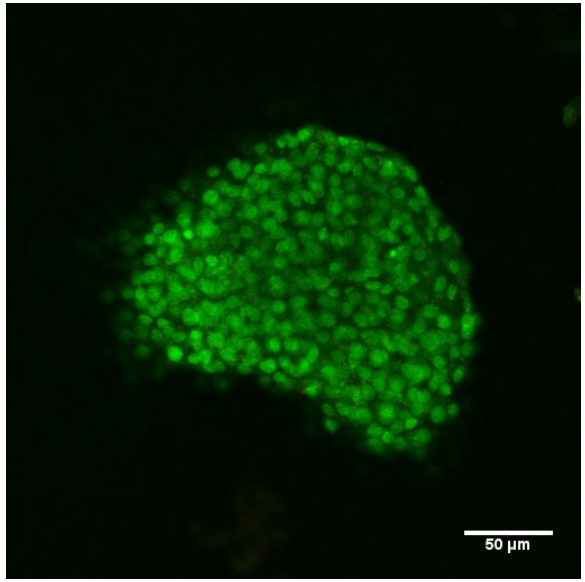


Non-treated islets

Ki67 -red

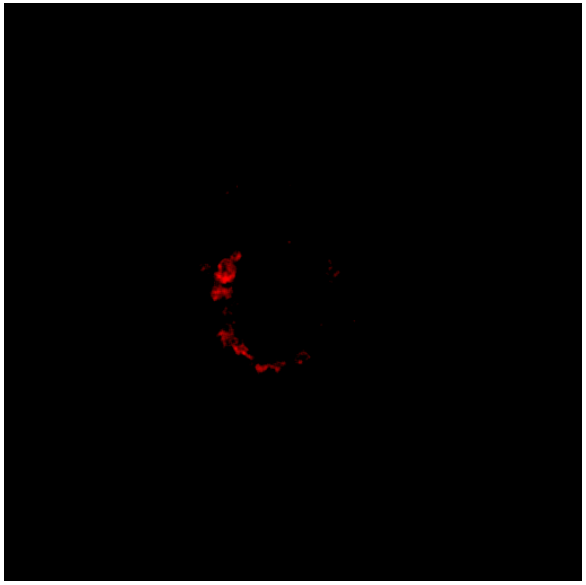


DAPI - green

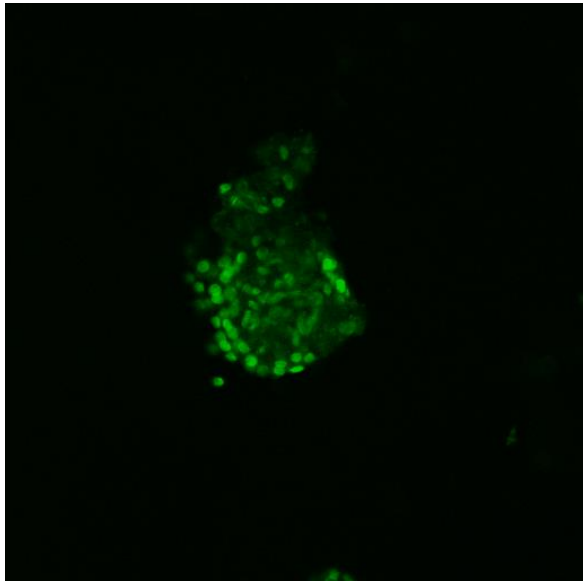


merge

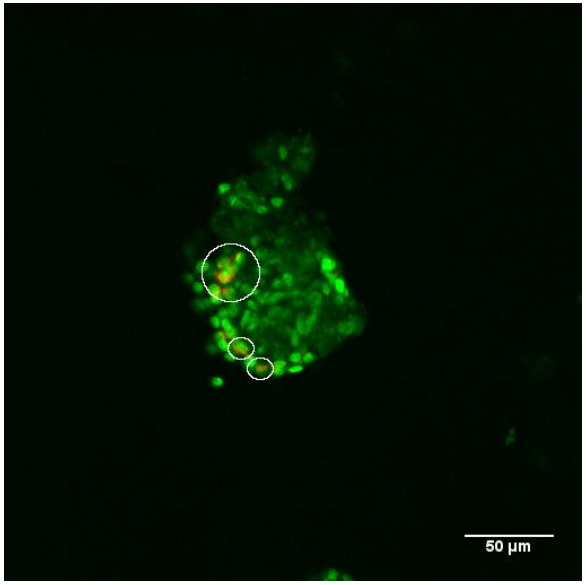
Islets treated with 100 µM GABA



Ki67 -red



DAPI - green



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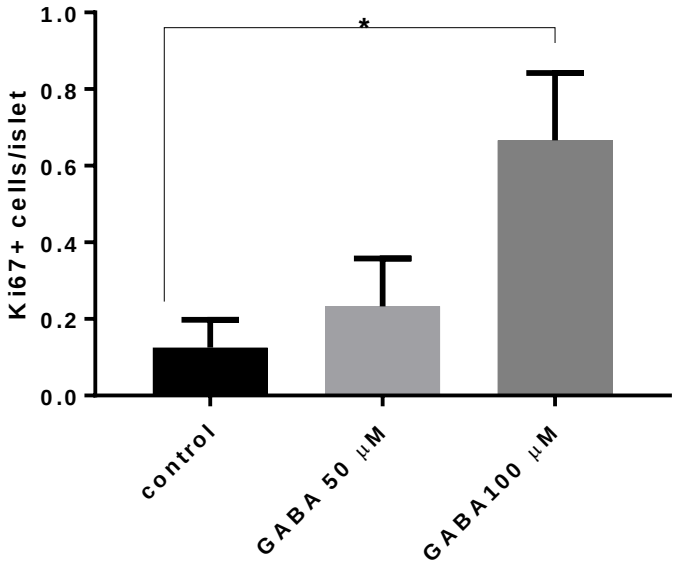
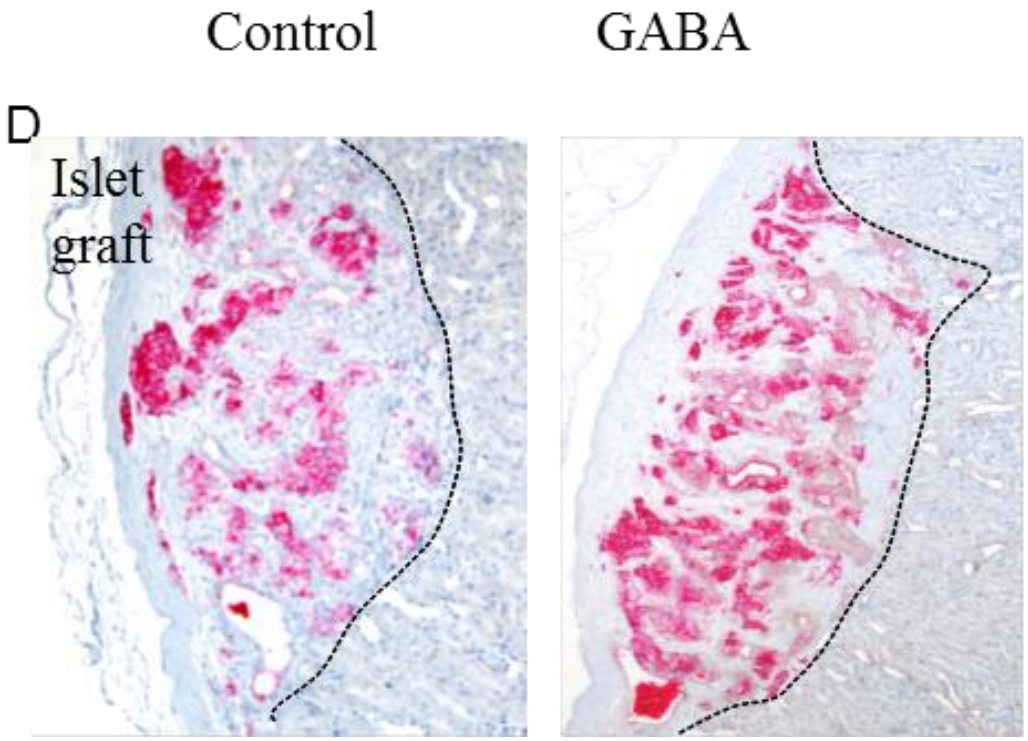
The effect of GABA on the proliferation of the cell in human pancreatic islets treated in vitro for 19 h.



Ki67 staining,
See images below

Human islets transplanted into immunodeficient mice: GABA stimulates growth (regeneration) of beta cells



HYPOTHESES

1. Combined therapy with GABA and a DPP-4 inhibitor (DPP4-I) will improve protection of human beta cells against injury/apoptosis and induce regeneration.
2. A completely oral therapy will be effective, which is a major clinical advantage.

GABA AND GLP-1 WORK TOGETHER

- Our most recent data show that GABA and GLP-1 are more effective when administered together.
- Improved beta-cell survival and regeneration.
- Effective on human beta cells.
- Completely oral therapy protects against diabetes in experimental model.

Streptozotocin-induced diabetes (MDSD)

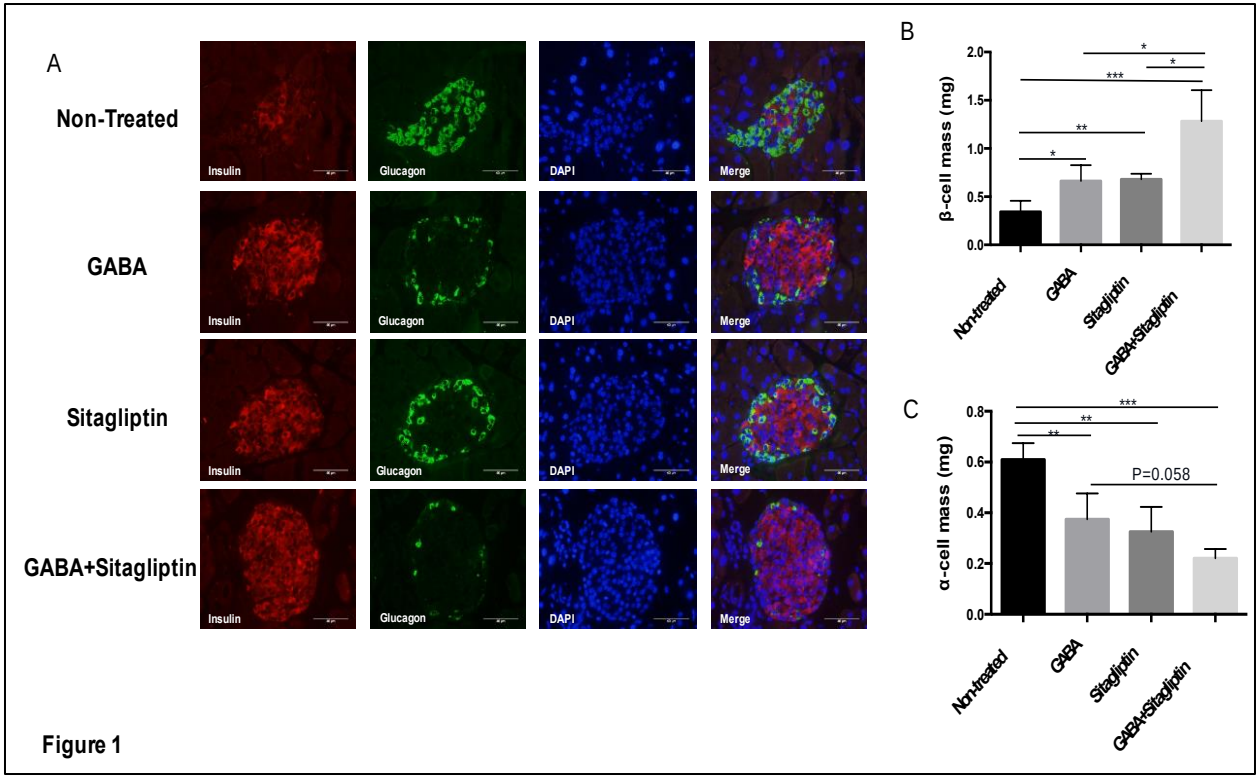
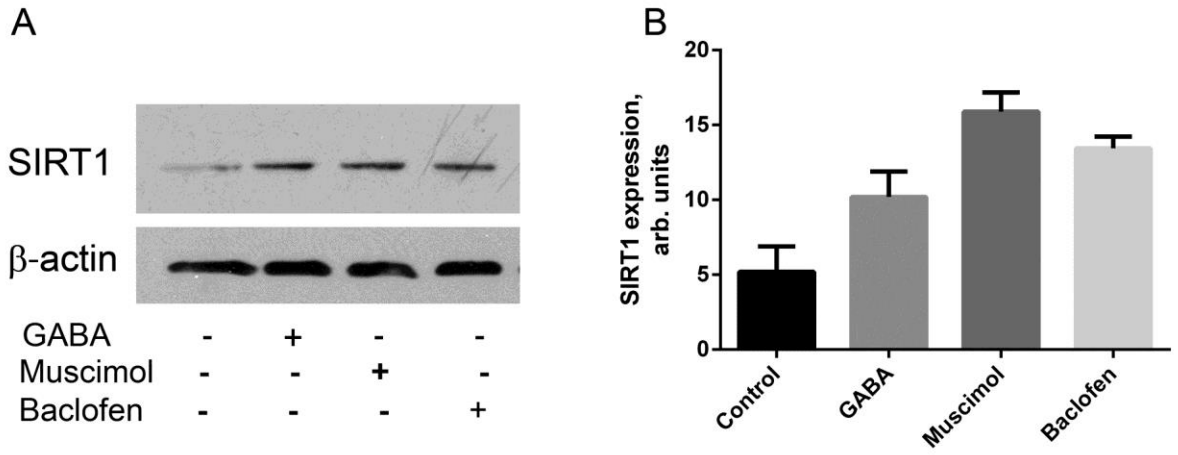


Figure 1

GABA AND DPP-4I collaborate to increase beta-cell mass. Red = insulin; green = glucagon; blue = DAPI. Treatment with combined drugs was superior to induce proliferation (Ki-67+ cells) and reduce apoptosis (Tunel assay); data not shown.

GABA and GABAergic drugs (agonistic) increase SIRT1 expression in INS-1 beta-cell line



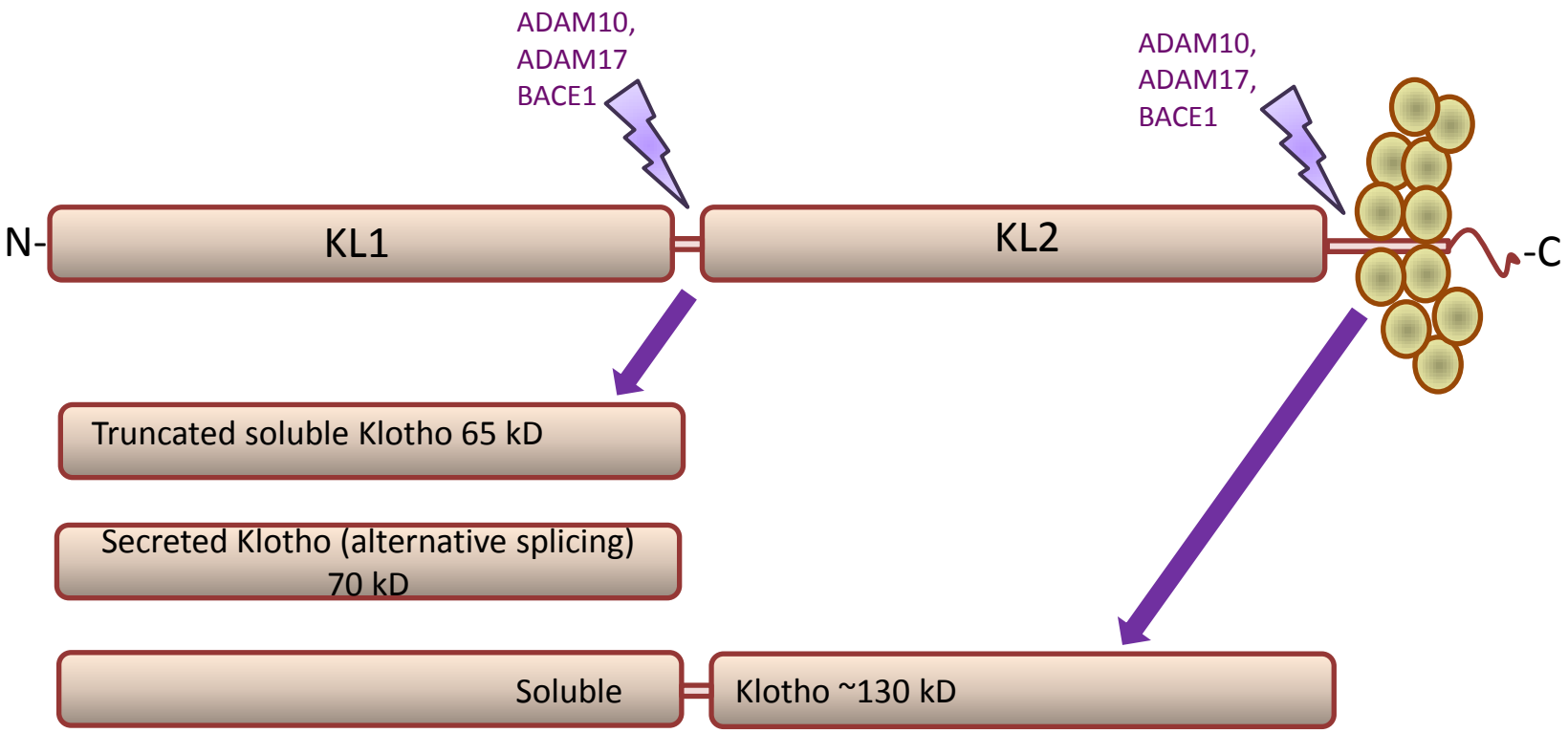
GABA increases Klotho (alpha-Klotho)

In humans Klotho normally declines with age, and is abnormally low in diabetic patients (type 1 and 2).

Klotho KO mice have multi-system disease and accelerated aging.

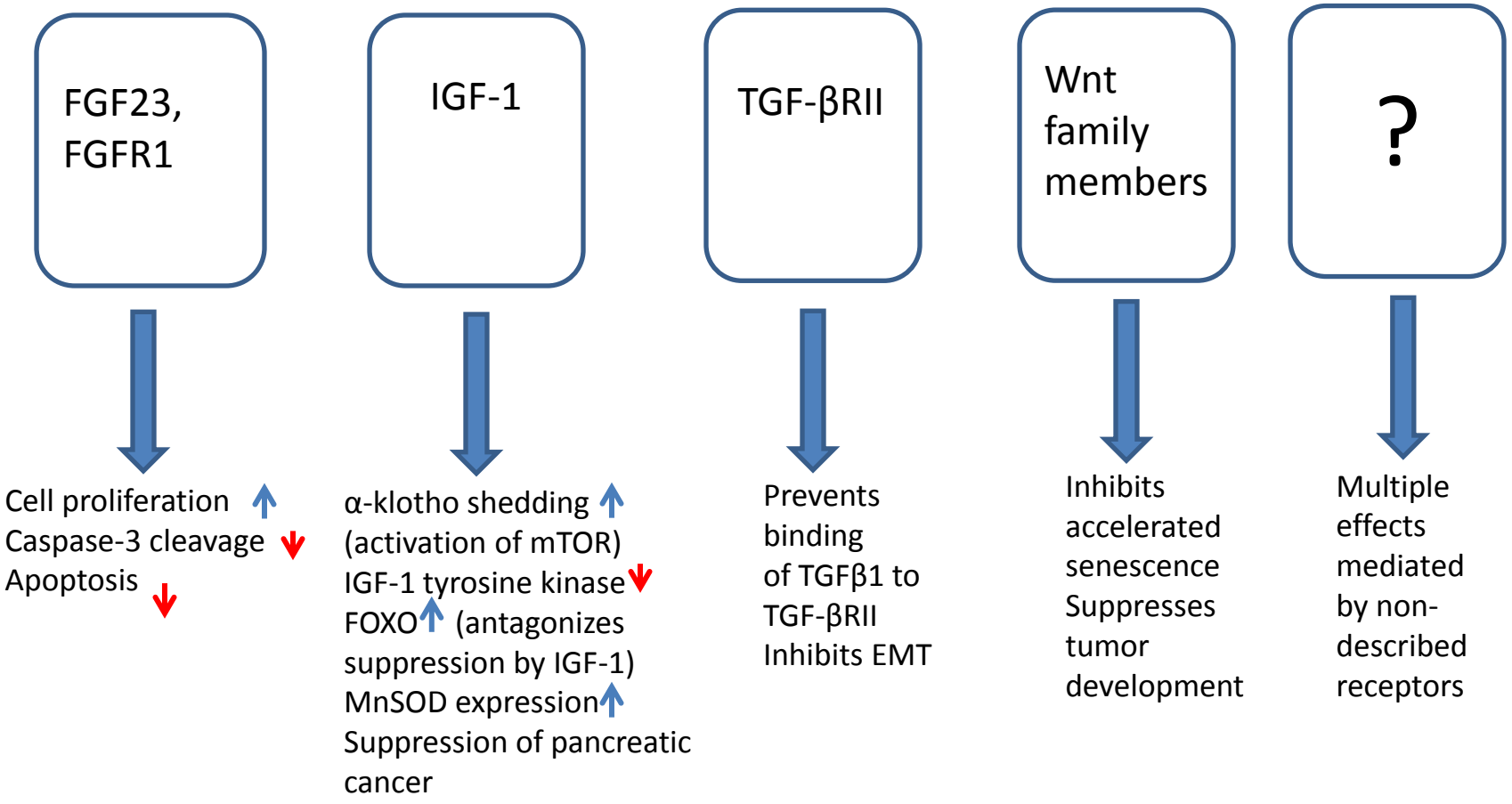
Klotho has multiple protective effects on beta cells. Importantly, it inhibits NF-kB activation and exerts anti-apoptotic effect.

Structure of α-Klotho



Membrane-bound klotho binds FGFR1 and facilitates binding of FGF23 to this receptor.
Soluble klotho acts as a hormone with the receptors yet to be characterized.
KL1 and KL2 domains structurally resemble glucosidase, but their enzymatic activity is questionable.

Binding partners of α-klotho



KLOTHO INHIBITS NF-κB ACTIVATION

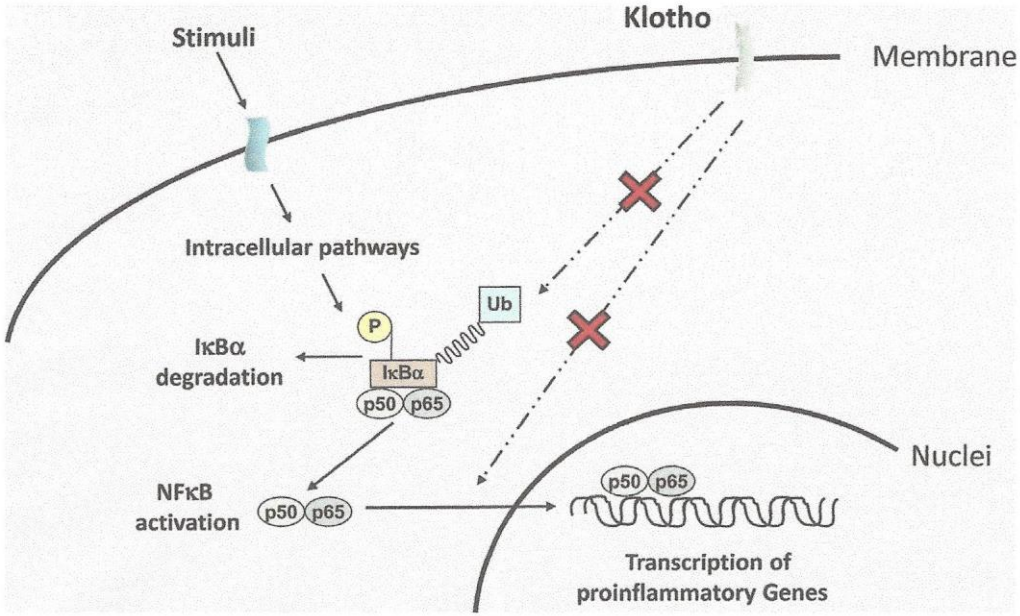
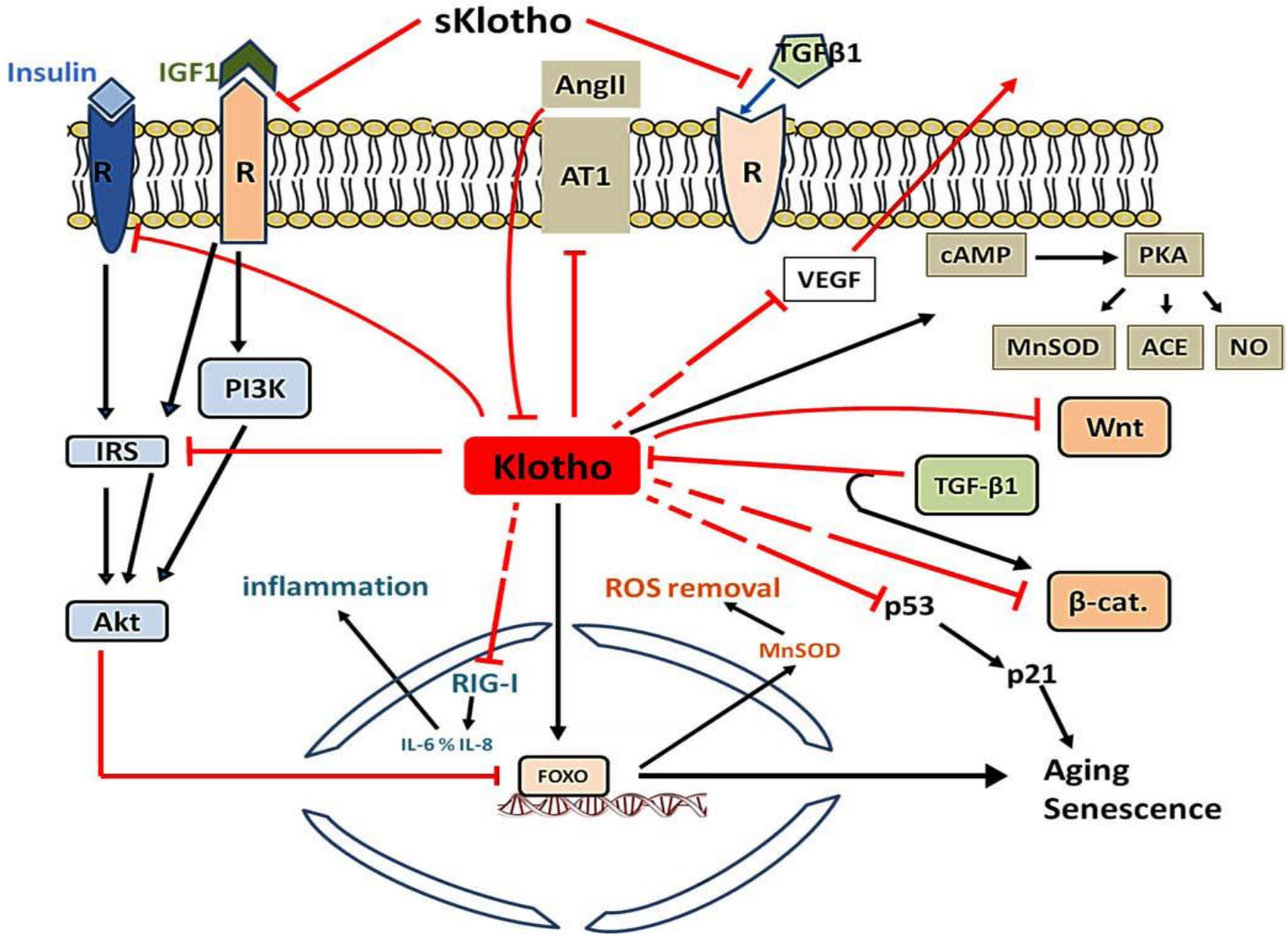


Fig. 4 Klotho and NFκB. Klotho suppresses nuclear NFκB activation and the subsequent transcription of proinflammatory genes.

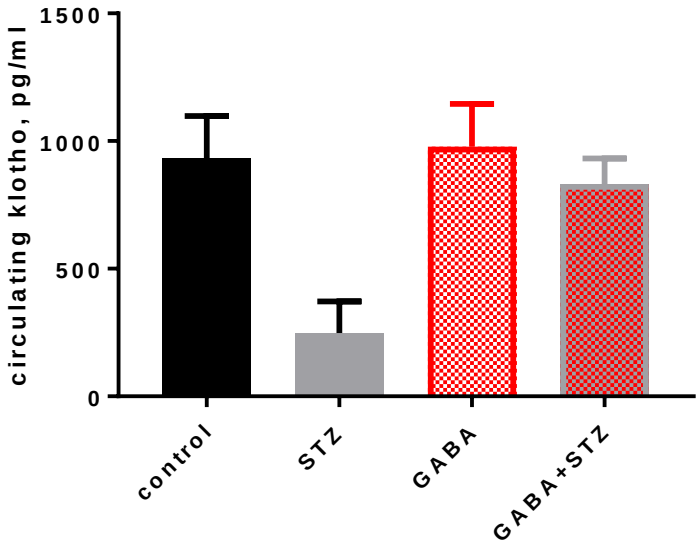
Buendia et al., Vitamins and Hormones, 2016, 101: 119-147



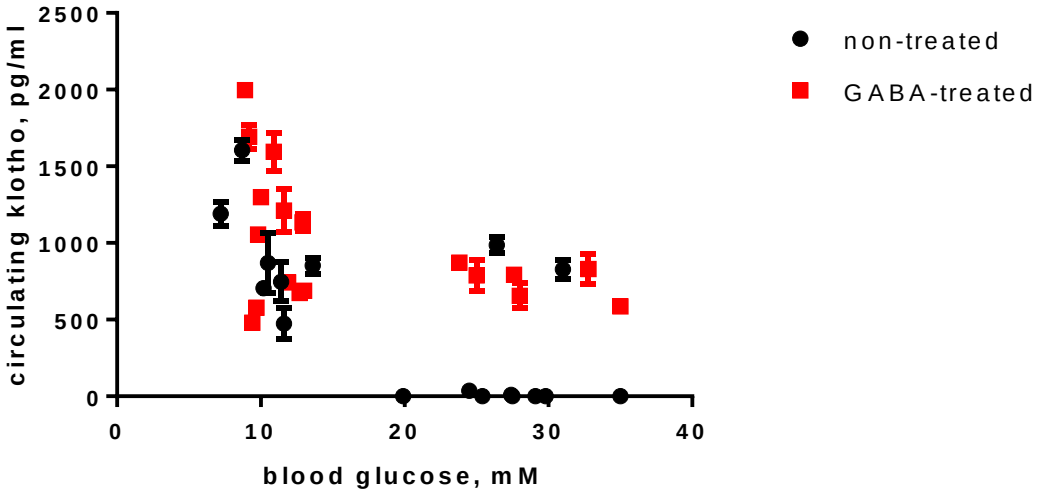
Schematic representation of the Klotho participating in intracellular signaling pathways. Klotho protein is involved in several intracellular signaling pathways that are essential for the regulation of many cellular processes, including aging and senescence.

From: ***M. Sopjani et al. Klotho and Intracellular Signaling Current Molecular Medicine, 2015, Vol. 15, No. 1*** **33**

The level of circulating klotho is decreased in diabetic C57 mice. It is restored by GABA in drinking water.

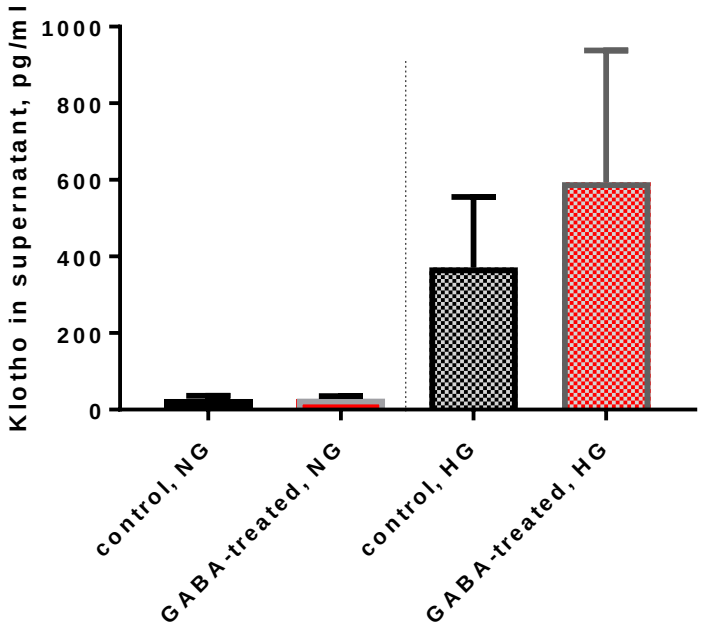


The level of circulating klotho negatively correlates with the level of blood glucose

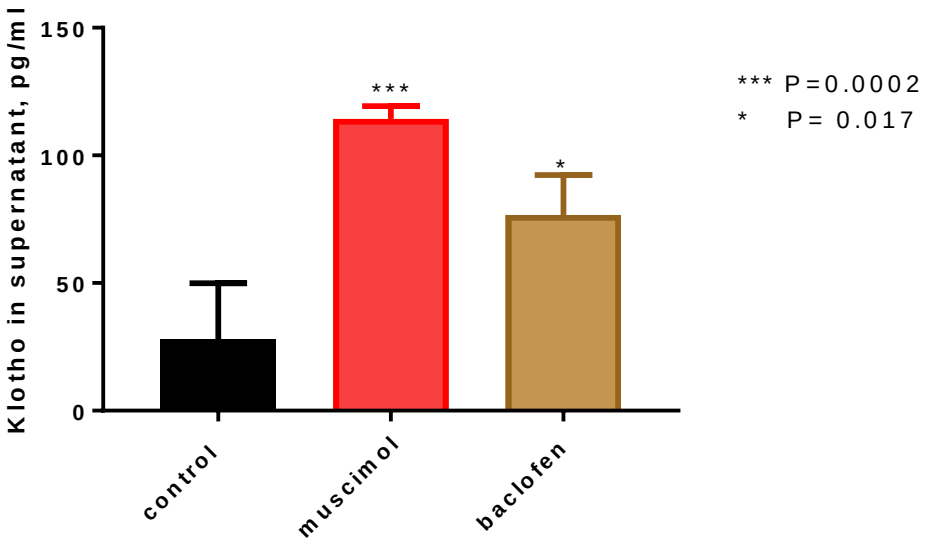


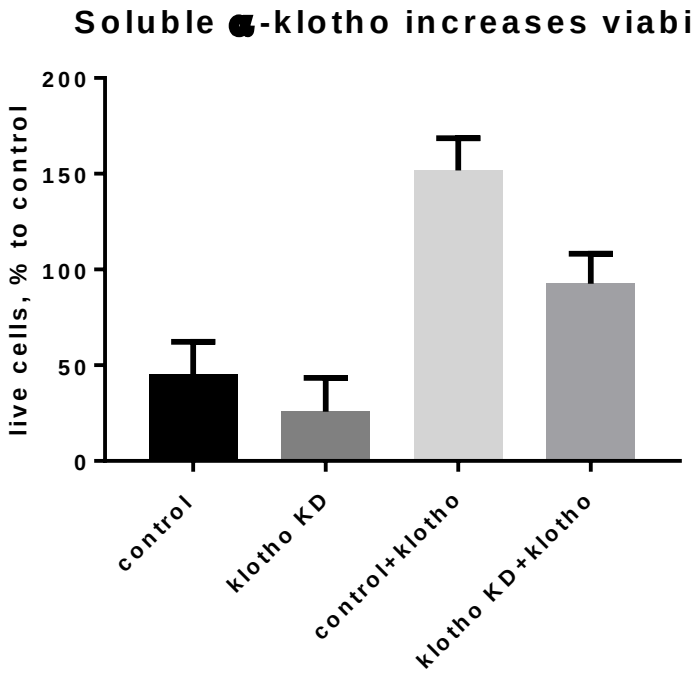
	blood glucose, mM vs. non-treated	blood glucose, mM vs. GABA-treated
Pearson r		
r	-0.6855	-0.4399
95% confidence interval	-0.8771 to -0.3055	-0.7522 to 0.034
R squared	0.4699	0.1935
P value		
P (one-tailed)	0.0012	0.0339
P value summary	**	*
Significant? (alpha = 0.05)	Yes	Yes
Number of XY Pairs	17	18

High Glucose induces release of Klotho by cultured human pancreatic islets.



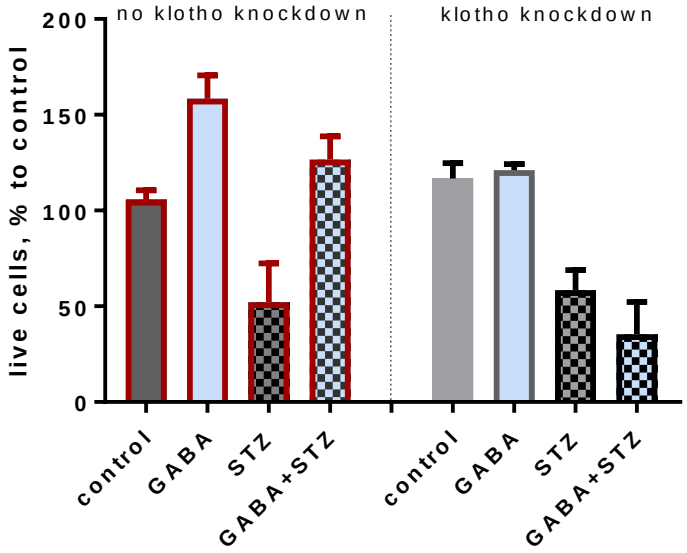
Cultured human pancreatic islets release Klotho. The release is notably activated by muscimol and baclofen.





Klotho knockdown
Recombinant Klotho Cell viability

Ability of GABA to increase cell viability and to protect INS-1 cells against STZ toxicity depends on α-klotho expression



Klotho knockdown
GABA
Cell viability

Effects of Klotho/our preliminary data:

1. Klotho is expressed by rat (INS-1) and human pancreatic cells (donor islets).
2. Cultured islet cells actively release soluble Klotho.
3. The level of circulating Klotho in mouse serum is severely decreased during STZ-induced diabetes. GABA, but not Liraglutide, restores it.
4. Ability of GABA to increase the survival of beta cells under stress depends on the expression of Klotho.

GABA in food and beverages: It is a safe natural compound

GABA INCREASES GROWTH HORMONE

SUMMARY: GABA has key effects against diabetes (studies performed with human cells)

1) It prevents beta-cell injury and death.
2) It promotes the regeneration of beta cells.
3) It suppresses immune cells that cause autoimmunity and beta-cell loss.
4) It increases SIRT1 and Klotho, which suppress NF-κB activation and exert protective effects on beta cells.

G. Prud'homme, Q. Wang and colleagues (PNAS 2011; Transplantation 2013; Diabetes 2014; BBRC 2014; Frontiers Pharmacology 2015, 2017)

Future Goals

1. Clinical development of a new treatment for type 1 and 2 diabetes: GABA therapy, with or without GLP-1.
2. Determine whether GABA therapy has a role in the treatment of other chronic diseases.

Research Team

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