Modulation of calcium signaling by chemogenetic tools to elucidate the pathogenesis of primary aldosteronism

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BACKGROUND

Primary aldosteronism (PA) is the most frequent form of secondary arterial hypertension with an estimate prevalence of 5-10% in the hypertensive patients. The identification of germline or somatic mutations in different genes coding for ion channels (*KCNJ5, CACNA1D*) and ATPases (*ATP1A1 and ATP2B3*) defines PA as a channelopathy. Although their role in aldosterone production is well understood, however their impact on proliferation and adrenal cortex nodulation remains to be established.

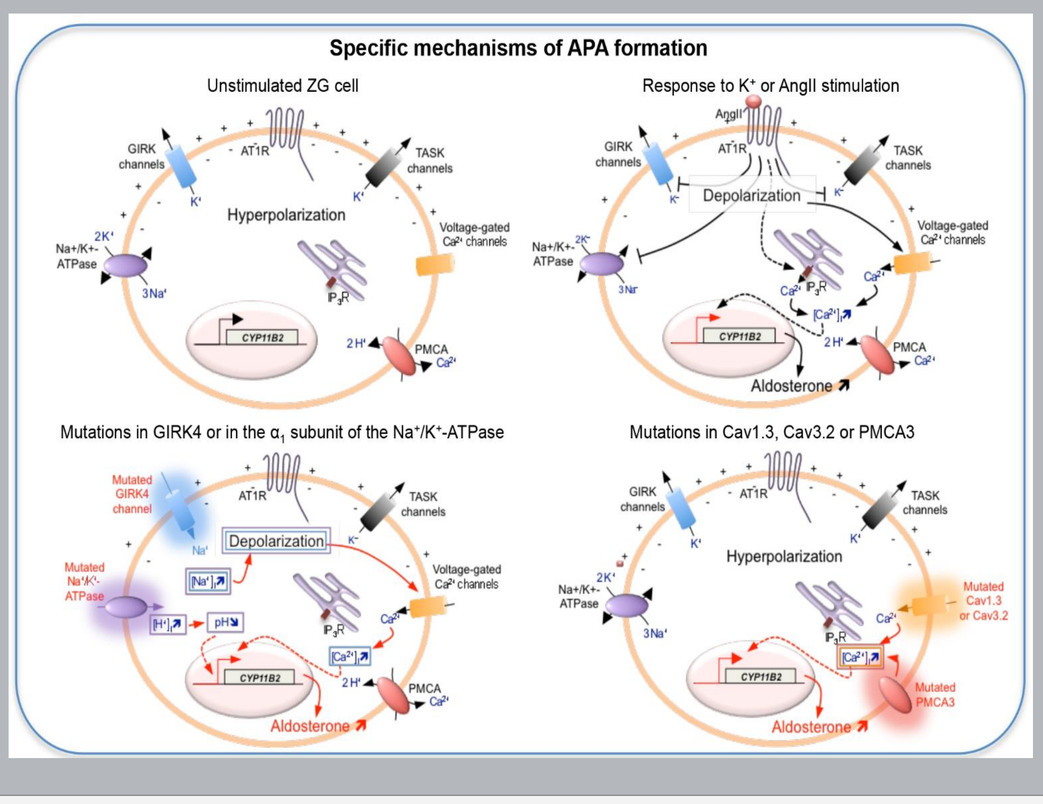

Figure 1. Regulation of aldosterone in normal and pathophysiological conditions. In basal conditions, zona glomerulosa (ZG) cells are in a hyperpolarized state due to the activity of potassium channels at the cell membrane. The binding of AngII to its receptor AT1R or the increase of extracellular K^+ concentration induce membrane depolarization. This depolarization leads to the opening of voltage gated calcium channels on the cell membrane increasing Ca^{2+} concentrations in the cytosol. Increased intracellular Ca^{2+} leads to the activation of calcium signaling pathway, the major trigger for aldosterone biosynthesis. In pathological conditions, mutations affecting specific ion channels and ATPases lead to a constitutively depolarized ZG cell membrane or directly to increased intracellular Ca^{2+} concentrations, constitutively activating Ca^{2+} signaling and therefore an autonomous aldosterone biosynthesis.

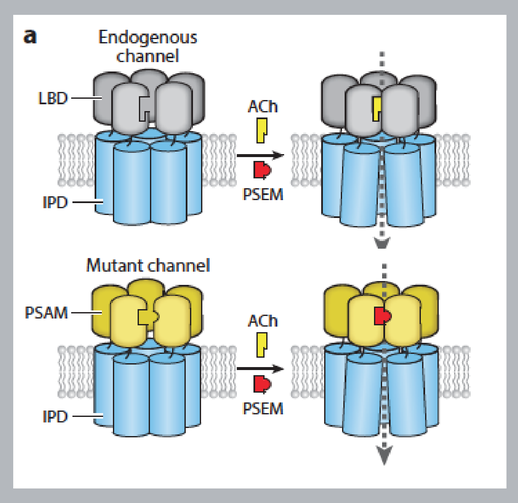

Figure 2. Chemogenetic tools. Pharmacologically Selective Actuator Modules (PSAMs) are modified ligand binding domains (LBDs) that are engineered to selectively interact with synthetic agonists called Pharmacologically Selective Effector Molecules (PSEMs). PSAMs can be combined with various ion pore domains (IPDs) from different ion channels to produce chimeric channels with common pharmacology but distinct functional properties.

OBJECTIVES

The aim of our study is to decipher mechanisms of aldosterone biosynthesis by modulating calcium signaling using chemogenetic tools.

MATERIALS AND METHODS

We have generated and characterized H295R_S2 stable cell line expressing a chimeric receptor (PSAM) allowing modulation of sodium entry into the cells in response to specific drugs by measuring intracellular calcium concentrations, cell proliferation and steroidogenic genes expression.

RESULTS

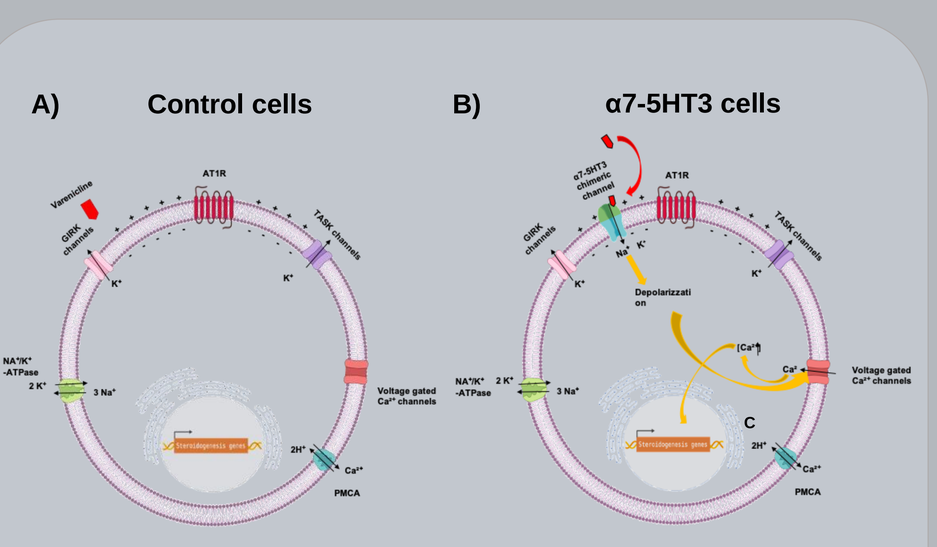

Fig.3. Effect of Varenicline on cells expressing empty vector (A, Control cells) or α7-5HT3 (B, α7-5HT3 cells) construct. **A)** In control cells, varenicline has no receptor and will not induce any change. **B)** In α7-5HT3 cells, varenicline binds to the α7-5HT3 receptor, leading to Na+ entry into the cells, cell depolarization, opening of voltage-gated Ca^{2+} channel and therefore an autonomous aldosterone production.

Dose-dependent effect of varenicline on intracellular Ca^{2+} concentrations

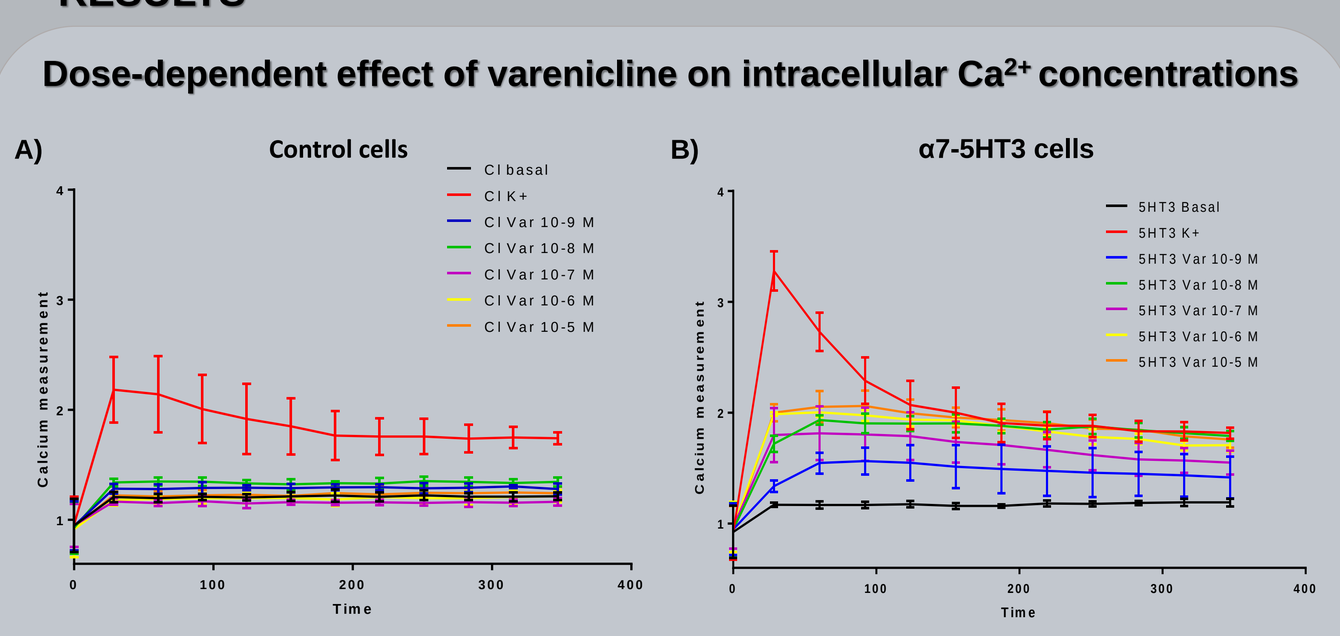

Fig.4. Dose-response effect of varenicline on intracellular Ca^{2+} concentrations in control and α7-5HT3 cells. Cells were treated with different concentrations of varenicline (10^{-9} to 10^{-5}M) or with K^+ (12mM)**.** Fura2 was used to determine intracellular Ca^{2+} concentration. Results are presented as 340/380 ratio. **A)** In control cells, treatment with different doses of varenicline does not modified the intracellular Ca^{2+} concentration whereas treatment with K^+ induces an increase of intracellular Ca^{2+} concentration. **B)** In α7-5HT3 cells, treatment with varenicline induces a dose-dependent increase of intracellular Ca^{2+} concentration. Treatment of α7-5HT3 cells with K^+ induces a strong increase of intracellular Ca^{2+} concentration. The results were analysed with Two Way ANOVA test.

Varenicline has no effect on cell proliferation

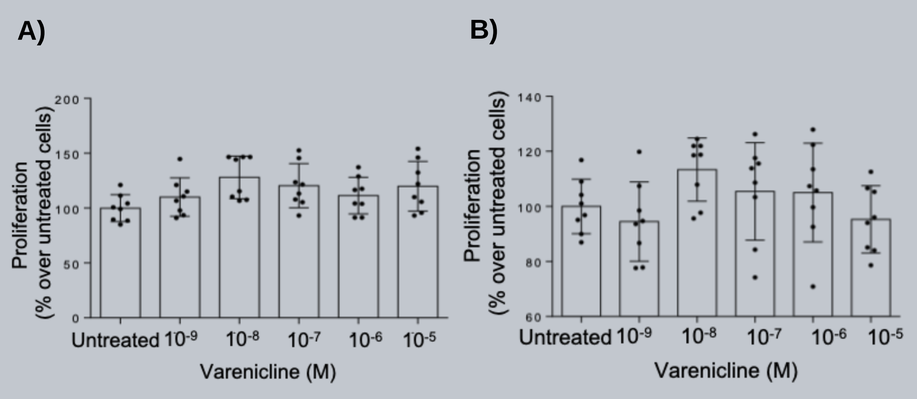

Fig.5. Dose-response effect of varenicline on cell proliferation in control and α7-5HT3 cells. Cells were treated with different concentrations of varenicline (10^{-9} to 10^{-5}M) or with K^+ (12mM)**.** Cell-Titer was used to determine cell proliferation. Results are presented as % over untreated cells. **A)** In control cells, treatment with different doses of varenicline does not modified cell proliferation. **B)** In α7-5HT3 cells, treatment with varenicline has no effect on cell proliferation. The results were analysed with Two Way ANOVA test.

Effect of varenicline on steroidogenic gene expression

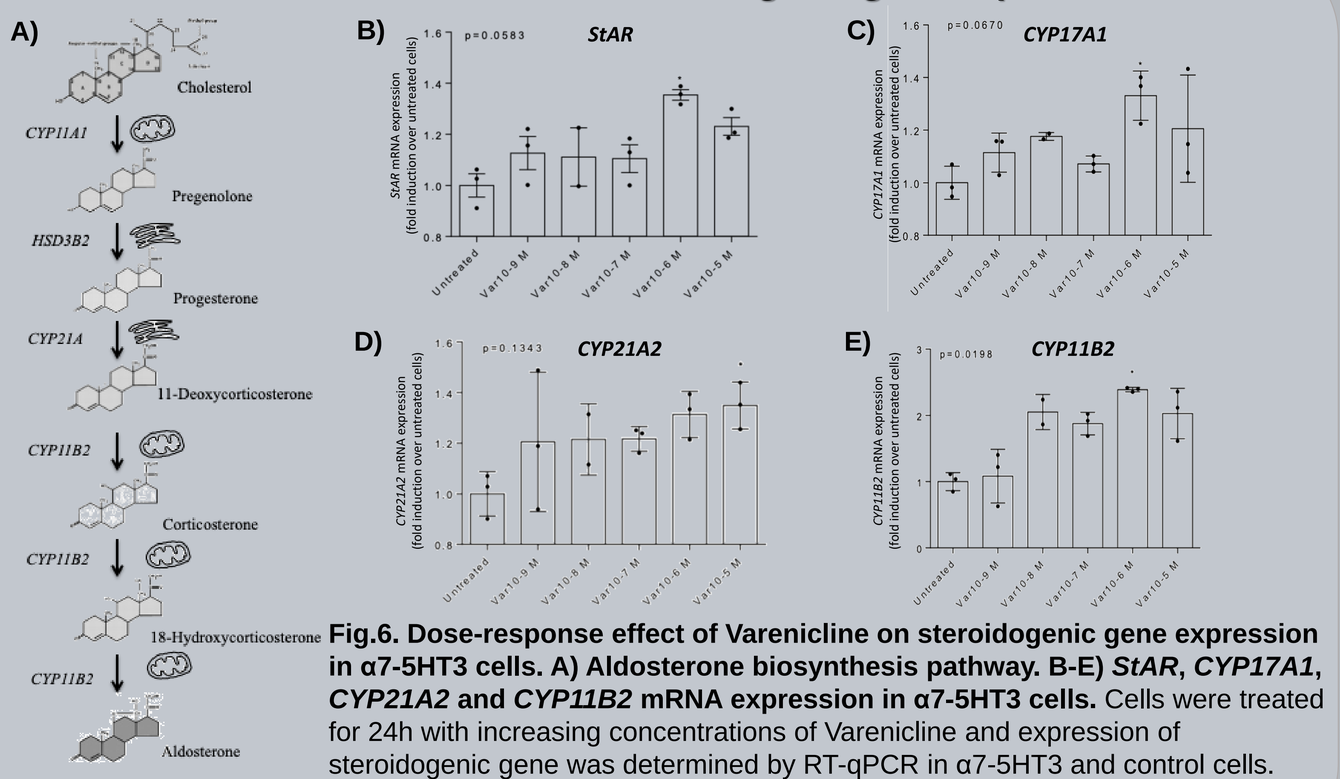

Fig.6. Dose-response effect of Varenicline on steroidogenic gene expression in α7-5HT3 cells. A) Aldosterone biosynthesis pathway. B-E) *StAR*, *CYP17A1*, *CYP21A2* and *CYP11B2* mRNA expression in α7-5HT3 cells. Cells were treated for 24h with increasing concentrations of Varenicline and expression of steroidogenic gene was determined by RT-qPCR in α7-5HT3 and control cells. Expression of *StAR, CYP17A1, CYP21A1* and CYP11B2 was increased only in α7-5HT3 cells. The results were analysed with Kruskal-Wallis test.

CONCLUSIONS

We generated a cell line in which we can modulate the intracellular calcium concentration « on demand ». This cell line will be a useful tool for a better understanding of the alterations of intracellular ion balance and calcium signaling in the pathophysiology of PA.