Identification of New Angiotensin Ⅱ Type 1 Receptor Interactions with β-arrestin Biased agonists and Angiotensin Receptor Blockers

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Identification of New Angiotensin II Type 1 Receptor Interactions with β-arrestin Biased agonists and Angiotensin Receptor Blockers

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【目的】

My Ph.D. thesis has two parts.

Part A: Ezrin, Radixin, and Moesin Phosphorylation in NIH3T3 Cells Revealed Angiotensin II Type 1 Receptor Cell-Type-Dependent Biased Signaling.

β-Arrestin biased agonists are a new class of drugs with promising therapeutic effects especially in the treatment of heart failure. β-Arrestin biased agonists were deeply investigated in cardiomyocytes and HEK 293 cells. They reduce apoptosis, improve cardiac functions and increase cardiomyocytes contractility. However, the molecular mechanisms of β-arrestin biased agonists in cardiac fibroblasts and fibroblast cell lines are still not completely identified. The purpose of this study is to investigate the effects of angiotensin II (AngII) and [Sar1,Ile4,Ile8] AngII (SII), a β-arrestin biased agonist, on ezrin-radixin-moesin (ERM) phosphorylation in NIH3T3 cells (a fibroblast cell line) stably expressing AngII type 1A receptor (AT1aR).

Part B: Angiotensin Receptor Blockers Show Distinct Effects on the Interaction of Angiotensin II Type 1 Receptor with G proteins and β-Arrestins.

AT1aR shows a basal activity which can be blocked by various AT1aR blockers (ARBs). The blockers that decrease basal activity are named as inverse agonists. Clinical investigations show distinct effects of these ARBs on reducing hypertension and controlling renal failure. In this study, we try to correlate these functional differences of ARBs with their specific interaction with G proteins and β-arrestins.

【方法】

NIH3T3 cells stably AT1aR were used in all experiments. For Western blot, the cells were transfected with plasmids or siRNA, and stimulated 48 h after transfection. Then, cell lysates were prepared and separated by SDS-polyacrylamide gel electrophoresis. Proteins in the gels were transferred

to polyvinylidene difluoride membranes, and was blotted with anti-pERM polyclonal antibody (Ab), anti-ERK monoclonal Ab, anti-pERK Ab, anti-β-arrestin Ab, anti-β-arrestin2 monoclonal Ab, anti-GAPDH Ab, or anti-αSMA Ab. Specific bands were detected using chemiluminescence Western blotting detection reagents. For bioluminescence resonance energy transfer (BRET) assay, the cells were plated in 100 mm tissue culture dishes and were transfected with various plasmids using X-treme Gene 9. The cells were used for BRET assay experiment 48 h after transfection.

【結果】

Part A: ERM Phosphorylation in NIH3T3 Cells Revealed Angiotensin II Type 1 Receptor Cell-Type-Dependent Biased Signaling.

1. ERM and ERK phosphorylation by AngII and SII stimulations.

AngII stimulated ERM phosphorylation at 5, 15, and 30 min stimulations, while SII did not. In contrast to ERM phosphorylation, AngII and SII stimulated ERK phosphorylation. AngII stimulated ERK phosphorylation in a graded manner with maximum response after 30 min. SII effect on ERK phosphorylation was rapid, sharp and transient with maximum response after 5 min. The effects of AngII and SII on intracellular Ca^{2+} increase were next examined. AngII but not SII stimulated the increase in intracellular Ca^{2+}. AngII-stimulated intracellular Ca^{2+} increase was sharp and transient. These results suggest that AngII stimulates Gq-phospholipase C pathway, but SII does not activate phospholipase C.

2. AngII-stimulated ERM phosphorylation is independent of Gαq.

The role of Gαq protein in AngII-stimulated ERM phosphorylation was examined by 3 interventions: (1) Over-expression of Gαq carboxyl terminal peptide (Gq-CT) which can inhibit Gαq coupling with AT1aR. (2) Over-expression of a constitutive active mutant of Gαq (Gq (Q209L)) which desensitizes AT1aR and down regulates Gαq downstream signals. (3) Over-expression of Gαq dominant negative mutant (Q209L/D277N) (DN-Gq). Gq-CT and DN-Gq did not affect AngII-stimulated ERM phosphorylation. Gq (Q209L) increased both basal and AngII-stimulated ERM phosphorylation. These results indicate that Gαq signaling inhibits ERM phosphorylation. These results also indicate that AngII-stimulated Gαq signaling in NIH3T3 cells is too weak to inhibit ERM phosphorylation.

3. AngII-stimulated ERM phosphorylation is dependent of β-arrestin2.

The roles of β-arrestin1 and β-arrestin2 on AngII-stimulated ERM phosphorylation were examined. Over-expression of β-arrestin1 significantly reduced ERM phosphorylation. However, over-expression of β-arrestin2 significantly increased both basal and AngII-stimulated ERM phosphorylation. The role of β-arrestin2 in AngII-stimulated ERM

phosphorylation was confirmed by knockdown experiment of β-arrestin2. These results indicate that AngII-stimulated ERM phosphorylation is dependent on β-arrestin2 and AngII acts as a β-arrestin biased agonist in NIH3T3 cells.

4. SII inhibits ERM phosphorylation as a Gαq biased agonist.

The effects of β-arrestin2 and Gαq on SII-ERM signaling were examined. Over-expression of β-arrestin2 significantly increased basal phosphorylation of ERM (pERM) proteins. However, SII-stimulated ERM phopshorylation was not affected by β-arrestin2 over-expression. In contrast, over-expression of Gq-CT and DN-Gq significantly increased SII-stimulated ERM phosphorylation. Over-expression of Gq (Q209L) significantly increased both basal and SII-stimulated ERM phosphorylation. These results indicate that SII stimulates AT1aR coupling with Gαq. These results also show that SII-mediated Gαq signaling is more powerful than β-arrestin signaling. Thus, SII acts as a Gαq biased agonist in NIH3T3 cells.

Part B: Angiotensin Receptor Blockers Show Distinct Effects on the Interaction of Angiotensin II Type 1 Receptor with G Proteins and β-Arrestins.

1. Effects of AngII, valsartan, irbesartan and losartan on the interaction of Gαq, Gαi and Gαs with Gγ2.

The interactions between G protein α and βγ subunits are detected using BRET technique. AngII, valsartan, and losartan significantly decreased the interaction between Gαq and Gγ2. On the other hand, irbesartan slightly increases the interaction. While all ARBs did not significantly stimulate the interaction of Gαi, and Gαs with Gγ2. These results suggest that some ARBs enhances the interaction between Gαq and Gγ2 subunits.

2. Effects of AngII, valsartan, irbesartan, and losartan on the interaction of AT1aR with β-arrestin2.

AngII, valsartan, irbesartan, and losartan significantly increase the interaction of AT1aR with β-arrestin2. This suggests that AngII, valsartan, irbesartan, and losartan promote the interaction of AT1aR with β-arrestin2.

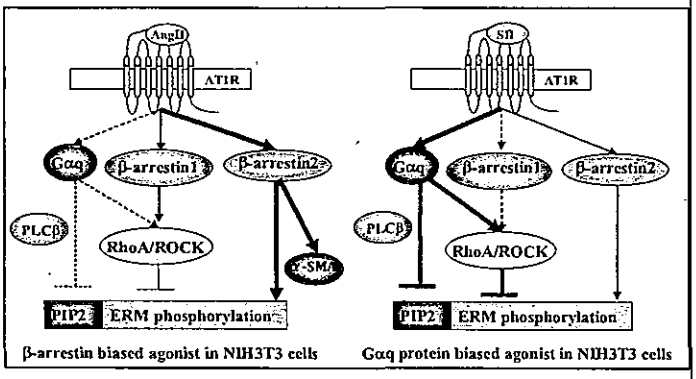
3. Effects of AngII, valsartan, irbesartan, and losartan on β-arrestin2 conformational changes.

AngII and various ARBs significantly induced the conformational changes in β-arrestin2. However, conformational changes by AngII and valsartan were different from those by irbesartan and losartan.

【考察】

This study demonstrated that AngII but not SII stimulates ERM phosphorylation in NIH3T3 cells stably over-expressing AT1aR. AngII-stimulated ERM phosphorylation in NIH3T3 cells is independent of

Gαq and β-arrestin1 but dependent on β-arrestin2. AngII can stimulate Gαq-mediated pathway, and Gαq and β-arrestin1 work together to antagonize β-arrestin2-mediated ERM phosphorylation (as shown in scheme). However, As AngII strongly activates β-arrestin2-mediated signal, AngII stimualtion overcomes these Gαq- and β-arrestin1-mediated inhibition. In contrast to AngII, SII activates Gαq-dependent signaling stronger than β-arrestin-dependent signaling in NIH3T3 cells. Thus, SII acts as a Gαq biased agonist in NIH3T3 cells. Unfortunately, we could not detect Gαq-mediated Ca^{2+} increase by SII stimulation. However, SII-mediated Gq activation was detected with BRET assay. Then, SII may interact with Gq but not induce the conformation that can activate phospholipase C. This study also shows that NIH3T3 cells are a good system to detect AT1aR-mediated biased agonism.



β-arrestin biased agonist in NIH3T3 cells　　Gαq protein biased agonist in NIH3T3 cells

Protein-protein interaction is essential for cellular signaling and detected by imaging techniques such as BRET or fluorescent resonance energy transfer (FRET) assays. In this study, BRET assay was used to examine whether different ARBs interact with G proteins and β-arrestin2 in drug-dependent manner. It was found that valsartan, irbesartan and losartan stimulate AT1aR interaction with β-arrestin2. Although the signals are low, valsartan and losartan may promote the dissociation of Gαq and Gβγ from heterotrimeric Gq. Furthermore, valsartan stimulated β-arrestin2 conformational change, while irbesartan and losartan did not. Thus, each ARB has its own unique and characteristic effects on AT1aR, G proteins, and β-arrestins interactions. This may give a good explanation for their distinct in vivo effects of ARBs on controlling hypertension, heart and renal failure. However, future studies are necessary to correlate the effects of ARBs between in vitro and in vivo.

【発表論文】

(1) Ibrahim IA, Nakaya M, Kurose H.: Ezrin, radixin, and moesin phosphorylation in NIH3T3 cells revealed angiotensin II type 1 receptor cell-type-dependent biased signaling. Journal of Pharmacological Sciences 118, 408 - 412 (2012)

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