Archives of Biochemistry and Biophysics 646 (2018) 24–30

Contents lists available at ScienceDirect

Archives of Biochemistry and Biophysics

journal homepage: www.elsevier.com/locate/yabbi

Cholesterol modulates the binding properties of human relaxin family peptide receptor 3 with its ligands

Jia-Hui Wang[1], Meng-Jun Hu[1], Xiao-Xia Shao, Dian Wei, Ya-Li Liu, Zeng-Guang Xu, Zhan-Yun Guo*

Research Centre for Translational Medicine at East Hospital, School of Life Sciences and Technology, Tongji University, Shanghai, China

ARTICLE INFO

Keywords:
Cholesterol
GPCR
Interaction
Ligand binding
Relaxin-3
RXFP3

ABSTRACT

Relaxin family peptide receptor 3 (RXFP3) is implicated in the regulation of food intake and stress response upon activation by its cognate agonist relaxin-3. As an A-class G protein-coupled receptor, RXFP3 is an integral plasma membrane protein with seven transmembrane domains, yet influence of the membrane lipids on its function remains unknown. In the present study, we disclosed that cholesterol, an essential membrane lipid for mammalian cells, modulated the binding properties of human RXFP3 with its ligands. We first demonstrated that depletion of cholesterol from host human embryonic kidney (HEK) 293T cells by methyl-β-cyclodextrin altered ligand-binding properties of the overexpressed human RXFP3, such as increasing its binding potency with some antagonists and decreasing its binding affinity with a NanoLuc-conjugated R3/I5 tracer. Thereafter, we demonstrated that two B-chain residues, B5Tyr and B12Arg, were primarily responsible for the increased binding potency of these antagonists with human RXFP3 under the cholesterol depletion condition. Our results suggest that cell membrane cholesterol interacts with human RXFP3 and modulates its ligand-binding properties, providing new insights into the influence of membrane lipids on RXFP3 function.

1. Introduction

Relaxin family is a group of peptide hormones, including relaxin (primates express two relaxins, namely relaxin-1 and relaxin-2), relaxin-3 (also known as INSL7), and insulin-like peptide 3–6 (INSL3–6) [1–4]. The relaxin family is a branch of the insulin superfamily that also includes insulin and insulin-like growth factor 1 and 2 (IGF-1 and IGF-2). The relaxin family peptides play a variety of biological functions, such as regulation of reproduction, food intake, stress response, and glucose homeostasis [1–4]. So far, four formerly orphan G protein-coupled receptors (GPCRs) have been identified as relaxin family peptide receptors, namely RXFP1–4. Relaxin and INSL3 are the cognate agonists of the homologous RXFP1 and RXFP2, respectively [5,6]. Relaxin-3 and INSL5 are the cognate agonists of the homologous RXFP3 and RXFP4, respectively [7,8]. In addition, relaxin-3 has also been shown to activate RXFP1 and RXFP4 *in vitro* with high efficiency [9,10].

The neuropeptide relaxin-3 is predominantly expressed by specific neurons in the nucleus incertus of the brain [11–19]. The mature relaxin-3 is composed of two polypeptide chains (an A-chain and a B-chain) with two interchain disulfide bonds and one intra-A-chain disulfide bond (Fig. 1A). It folds into a globular structure similar to that of other relaxin family peptides (Fig. 1B), but its B-chain C-terminus adopts a folded-back conformation in solution, probably due to high flexibility of its B23Gly and B24Gly residues [20]. Besides the endogenous agonist relaxin-3, some synthetic agonists, such as the chimeric R3/I5 and stapled B-chain analogues, have been designed for RXFP3 [21–23]. As shown in Fig. 1A, the two-chain chimeric R3/I5 peptide contains the B-chain of relaxin-3 and the A-chain of INSL5. It folds into a tertiary structure similar to that of relaxin-3 and retains full binding and activation potencies at RXFP3 [21,24], suggesting that the B-chain of relaxin-3 is mainly responsible for binding and activation of this receptor. Some antagonists have also been designed for RXFP3, such as the chimeric R3(BΔ23–27)R/I5 and [G(B23)A,G(B24)S]R3/I5, as well as the shortened B-chain analogue R3B1–22R [25–28]. As shown in Fig. 1A, the two-chain antagonist R3(BΔ23–27)R/I5 has a shortened B-chain C-terminus (deletion of B23–27) and an additional C-terminal Arg residue [25]. In contrast, the two-chain antagonist [G(B23)A,G(B24)S]R3/I5 has a rigid B-chain C-terminus, which is obtained by replacement of B23Gly and B24Gly of R3/I5 with the corresponding Ala–Ser dipeptide of human INSL5 [26]. Both R3(BΔ23–27)R/I5 and [G(B23)A,G(B24)S]R3/I5 retain high binding potency with RXFP3, but completely lose activation potency toward this receptor,

* Corresponding author.
E-mail address: zhan-yun.guo@tongji.edu.cn (Z.-Y. Guo).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.abb.2018.03.031
Received 20 January 2018; Received in revised form 10 March 2018; Accepted 26 March 2018
Available online 27 March 2018
0003-9861/

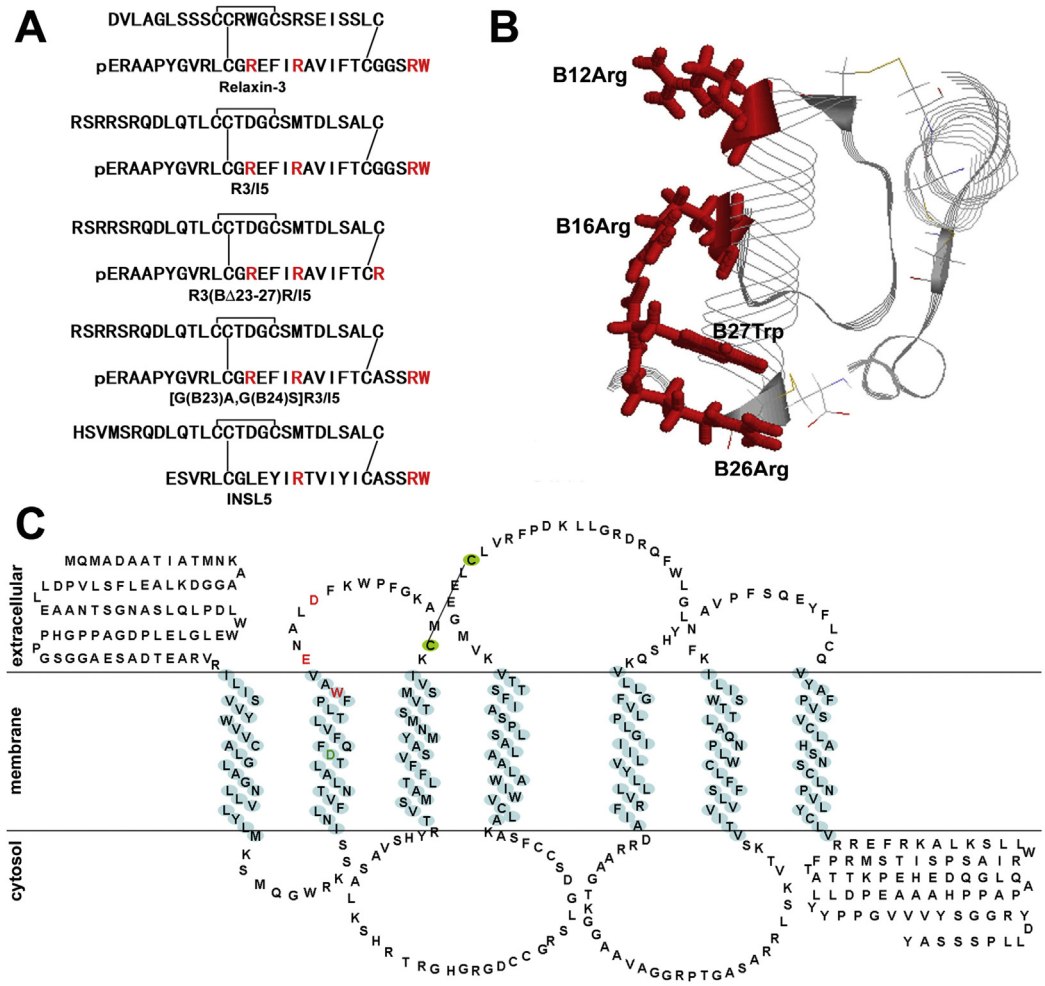

Fig. 1. (A) Amino acid sequences and disulfide linkages of some RXFP3 ligands used in the present study. The key B-chain Arg and Trp residues that interact with the WxxExxxD motif of RXFP3 are shown in red. Disulfide linkages are shown as lines. **(B)** The previously solved solution structure of human relaxin-3 (PDB ID: 2FHW). The key B-chain Arg and Trp residues that interact with the WxxExxxD motif of RXFP3 are shown as red sticks. **(C)** The predicted transmembrane topology of human RXFP3. The WxxExxxD motif at the extracellular end of TMD2 is shown in red. The essential negatively charged Asp residue in the middle of TMD2 is shown in green.

suggesting that either the length or conformation of the B-chain C-terminus of R3/I5 can affect RXFP3 activation.

Relaxin family peptide receptor 3 (RXFP3) is an A-class GPCR predominantly expressed by specific neurons in the brain [11–18]. It is implicated in the regulation of food intake, stress response, arousal, and exploratory behaviors upon activation by relaxin-3 [11–18]. As shown in Fig. 1C, RXFP3 has seven predicted transmembrane domains (TMDs) with an extracellular N-terminus and an intracellular C-terminus. Recent studies have disclosed a highly conserved essential WxxExxxD motif at the extracellular end of TMD2 of RXFP3 (Fig. 1C, shown in red). This motif forms electrostatic and hydrophobic interactions with the positively charged B-chain Arg residues (B12Arg, B16Arg, and B26Arg) and the large aromatic B27Trp residue of relaxin-3 [29–31]. A negatively charged aspartate residue (Fig. 1C, shown in green) in the middle of TMD2 is essential for transduction of the extracellular ligand-binding information to the intracellular region of RXFP3 to initiate downstream signaling [32].

All GPCRs are integral plasma membrane proteins with seven TMDs; thus, cell membrane lipids, such as cholesterol, might affect their functions. However, so far only a few GPCRs, including the oxytocin receptor [33–35], cholecystokinin receptors [36–38], and serotonin receptors [39–41], have been reported to be functionally affected by cholesterol [42–44]. In the present study, we disclose that cell membrane cholesterol modulates the binding properties of human RXFP3 with its ligands, and thus provide new insights into the influence of membrane lipids on the function of RXFP3 for the first time.

2. Experimental methods

2.1. Preparation of peptides and bioluminescent tracers

The two-chain relaxin family peptides used in the present work were prepared through overexpression of single-chain precursors in *Escherichia coli* and subsequent *in vitro* refolding and enzymatic maturation according to our previous procedures [26,31,45–48]. The mature peptides were purified to homogeneity by high performance liquid chromatography (HPLC) using a C18 reverse-phase column (Zorbax 300SB-C18, 4.6 mm × 250 mm, from Agilent Technologies, Santa Clara, CA, USA) and confirmed by mass spectrometry. The bioluminescent tracers, R3/I5-Luc and R3(BΔ23–27)R/I5-Luc, were prepared through chemical conjugation with an engineered NanoLuc reporter according to our previous procedure [31].

2.2. Transfection and cholesterol depletion of HEK293T cells

Human embryonic kidney (HEK) 293T cells were cultured in complete medium (DMEM medium plus 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin) in a CO_2 incubator at 37 °C with normal passage. For transfection, HEK293T cells were seeded into 35 mm dishes and grown to ~80% confluence within 24–36 h. Cells