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Structural determinants governing β-arrestin2 interaction with PDZ proteins and recruitment to CRFR1

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ABSTRACT

β-Arrestins are multifunctional adaptor proteins best know for their vital role in regulating G protein coupled receptor (GPCR) trafficking and signaling. β-arrestin2 recruitment and receptor internalization of corticotropin-releasing factor receptor 1 (CRFR1), a GPCR whose antagonists have been shown to demonstrate both anxiolytic- and antidepressant-like effects, have previously been shown to be modulated by PDZ proteins. Thus, a structural characterization of the interaction between β-arrestins and PDZ proteins can delineate potential mechanism of PDZ-dependent regulation of GPCR trafficking. Here, we find that the PDZ proteins PSD-95, MAGI1, and PDZK1 interact with β-arrestin2 in a PDZ domain-dependent manner. Further investigation of such interaction using mutational analyses revealed that mutating the alanine residue at 175 residue of β-arrestin2 to phenylalanine impairs interaction with PSD-95. Additionally, A175F mutant of β-arrestin2 shows decreased CRF-stimulated recruitment to CRFR1 and reduced receptor internalization. Thus, our findings show that the interaction between β-arrestins and PDZ proteins is key for CRFR1 trafficking and may be targeted to mitigate impaired CRFR1 signaling in mental and psychiatric disorders.

1. Introduction

The Corticotropin-releasing factor receptors, CRFR1 and CRFR2, belong to secretin family of G protein-coupled receptors (GPCRs), couple $G_{\alpha s}$ and share 70% amino acid sequence homology, but have distinct cell and tissue expression patterns [1,2]. CRFR1 is mainly expressed in cerebral cortex, cerebellum, medial septum, and anterior pituitary while CRFR2 is mainly expressed in heart and skeletal muscle [1,3]. The neuropeptide CRF displays a ten-fold higher affinity for CRFR1 over CRFR2 [4] and is mainly released from hypothalamus in response to stressors to trigger the release of adrenocorticotropic hormone (ACTH) and increase blood cortisol levels [5,6]. Although CRF is crucial for coping with stress responses [7], elevated CRF levels has been shown to correlate with anxiety disorders and depression. This was supported by elevated CRF levels detected in post-mortem brains of depressed suicide victims [8–11]. Moreover, CRFR1 KO mice show an impaired stress response and CRFR1 antagonists have demonstrated anxiolytic and antidepressant-like effects [5,12–14]. Interestingly, stimulation of CRFR1 in both cell cultures and mouse prefrontal cortical neurons resulted in enhanced signaling of the serotonin 2A receptor (5-HT_{2A}R) [15], another GPCR that regulates anxiety and depressive-like behaviors [16]. Taken together, these findings suggest that CRFR1 can be a viable target for treatment of mood and anxiety disorders and further investigation of CRFR1-scafolding proteins that modulate signaling cascade and trafficking of the receptor is essential.

Postsynaptic density protein of 95 kilodaltons (PSD-95), disc large, zona occludens (PDZ) domain-containing proteins are one of the most abundant GPCR-interacting proteins and are important regulators of

Abbreviations: 5-HT_{2A}R, serotonin 2A receptor; ACTH, adrenocorticotropic hormone; CAL, cystic fibrosis transmembrane conductance regulator-associated ligand; CRF, corticotropin releasing factor; CRFR, Corticotropin-releasing factor receptor; ERK, extracellular signal-regulated kinase; GK, guanylate kinase–like; GPCR, G protein-coupled receptor; HA, hemagglutinin; HEK293, human embryonic kidney 293; MAGI, membrane-associated guanylate kinase protein; PDZ, PSD95/Disc Large/Zona Occluden; PDZK1, PDZ domain–containing kidney protein 1; PSD-95, Postsynaptic density protein of 95 kDa; RMSD, root mean square deviation; ROCK, Rho-associated coiled coil-forming kinase.; SAP97, synapse-associated protein 97; SH3, SRC homology 3; SNX27, PDZ protein sorting nexin 27; Vps26, Vacuolar protein sorting-associated protein 26

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