Molecular Mechanism of Regulation of Receptor Type-Specific Gq Signaling by RGS8

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RGS (regulators of G-protein signaling) proteins comprise a large family that modulates heterotrimeric G-protein signaling. RGS8 is a neuron-specific RGS protein, which belongs to the B/R4 subfamily composed of the short N-terminus and the RGS domain. We previously showed that RGS8 suppressed Gq signaling in a receptor type-specific manner. However, RGS8S, a splice variant with different N-terminus, showed a diminished effect. To approach the molecular mechanisms underlying receptor type-specific attenuation by RGS8, we investigated the possibility that RGS8 protein may directly interact with certain G-protein-coupled receptors. We found that RGS8 directly binds to the third intracellular (i3) loop of M1 and M3 muscarinic acetylcholine receptors (mAChRs) but not M2-mAChR, and binding of RGS8S was weaker. Using GST-fusion proteins of three parts of M1i3, we found that RGS8 bound to all three parts, whereas RGS8S could not bind to C-terminal part of M1i3. Moreover, the deletion of N-terminal 9 aa and substitution of both Arg-8 and Arg-9 for Ala of RGS8 resulted in the reduced binding to M1i3. Bioluminescence resonance energy transfer (BRET) experiments revealed that RGS8 actually interacts with M1-mAChR, but RGS8S does not in the living cells. Furthermore, RGS8 mutant with less binding ability to M1i3 showed reduced inhibitory function of Gq signaling through M1-mAChR. These results clearly demonstrated that RGS8 can directly interacts with M1-mAChR via its N-terminus and i3 loop of the receptor, and this binding must play a essential role in receptor specific suppression by RGS8 in the living cells.