Ubiquitin-mediated receptor degradation contributes to development of tolerance to MrgC agonist-induced pain inhibition in neuropathic rats

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Abstract

Background and Purpose: Agonists to subtype C of the Mas-related G-protein-coupled receptors (MrgC) induce neuropathic pain inhibition after intrathecal (i.t.) administration in rodent models of nerve injury. Here, we investigated whether tolerance develops after repeated drug treatments and examined the underlying mechanisms. Experimental Approach: In male rats at 4-5 weeks after an L5 spinal nerve ligation (SNL), we conducted behavior tests to examine whether pain inhibition by JHU58, a dipeptide MrgC agonist, diminishes after repeated administration. We then examined agonist-induced endocytosis of MrgC in HEK293T cells, and the role of receptor ubiquitination in tolerance to JHU58-induced pain inhibition. Key Results: The inhibition of mechanical and heat hypersensitivity by JHU58 (0.1 mM, 10 μL, i.t.) decreased in SNL rats after repeated treatments with 0.5 mM JHU58 (10 μL, i.t., twice/day for 3 days). In HEK293T cells, acute treatment with JHU58 or BAM8-22 (a large peptide MrgC agonist) led to MrgC endocytosis from the cell membrane, and later sorting to the membrane for reinsertion. However, chronic exposure to JHU58 increased the coupling of MrgC to β-arrestin-2 and led to the ubiquitination and degradation of MrgC. Importantly, co-treatment with TAK-243 (0.2 mM, 5 μL, i.t.), a small-molecule inhibitor of the ubiquitin activating enzyme, during tolerance induction attenuated the development of JHU58 tolerance in SNL rats. Conclusion and Implications: These findings suggest that tolerance can develop to MrgC agonist-induced pain inhibition after repeated intrathecal administrations. This tolerance development may involve increased coupling of MrgC to β-arrestin-2 and ubiquitin-mediated receptor degradation.

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