NEUTROPHIL FUNCTION AND SIGNALING INDUCED BY LIGANDS FOR THE FORMYL PEPTIDE RECEPTOR 2

Akademisk avhandling

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av André Holdfeldt

Fakultetsopponent: Professor Richard D Ye, The Chinese University of Hong Kong, Shenzhen, China

Avhandlingen baseras på följande delarbeten

I. André Holdfeldt, Agnes Dahlstrand Rudin, Michael Gabl, Zahra Rajabkhani, Gabriele M. König, Evi Kostenis, Claes Dahlgren, and Huamei Forsman, 2017, **Reactivation of Gαi-coupled formyl peptide receptors is inhibited by Gαq selective inhibitors when induced by signals generated by the PAF receptor,** *J Leukoc Biol.102(3):871-880.*

II. André Holdfeldt, Sarah Line Skovbakke, Michael Gabl, Christina Nielsen, Claes Dahlgren, Henrik Franzyk, and Huamei Forsman, 2019, **Structure–Function Characteristics and Signaling Properties of Lipidated Peptidomimetic FPR2 Agonists: Peptoid Stereochemistry and Residues in the Vicinity of the Headgroup Affect Function**, *ACS Omega 4 (3), 5968–5982*.

III. André Holdfeldt, Simon Lind, Camilla Hesse, Claes Dahlgren, Huamei, Forsman, 2020, **The PAR4-derived pepducin P4Pal10 lacks effect on neutrophil GPCRs that couple to Gαq for signaling but distinctly modulates function of the Gαi-coupled FPR2 and FFAR2**, *Biochemical Pharmacology 180, 114143*.

IV. Martina Sundqvist*, André Holdfeldt*, Shane C Wright, Thor C Møller, Esther Siaw, Karin Jennbacken, Henrik Franzyk, Michel Bouvier, Claes Dahlgren, Huamei Forsman, 2020, **Barbadin selectively modulates FPR2-mediated neutrophil functions independent of receptor endocytosis**, *Biochim Biophys Acta Mol Cell Res. 867(12):118849.* * These authors contributed equally.

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NEUTROPHIL FUNCTION AND SIGNALING INDUCED BY LIGANDS FOR THE FORMYL PEPTIDE RECEPTOR 2

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ABSTRACT

Neutrophil pattern recognition receptors belonging to the G-protein coupled receptor (GPCR) family play a role in the processes of initiation as well as resolution of inflammatory processes. Formyl peptide receptor 2 (FPR2) in neutrophils is such a receptor and plays an important role in inflammation.

This thesis focuses on the molecular basis for FPR2 ligand recognition, receptor signaling and activation of neutrophils. The experimental data generate new knowledge that is related specifically to FPR2 but also of general importance for GPCR function, knowledge possibly of importance also for future drug development. To characterize FPR2 mediated signaling, cell-based in vitro methods were used, including sensitive methods to measure i) production of reactive oxygen species (ROS), ii) the transient rise of intracellular calcium ions, iii) chemotactic migration, iv) β-arrestin recruitment and, v) the dynamic reorganization of the actin cytoskeleton.

A new class of FPR2 ligands belonging to peptide mimetics (peptidomimetics) were identified and characterized as functional selective (biased) agonists triggering ROS release but not chemotaxis, a neutrophil function linked to receptor recruitment of β-arrestin. A novel receptor crosstalk-signaling pathway is also disclosed, a pathway leading to a reactivation of desensitized FPRs and involve a Gαq containing G-protein downstream of the receptor for platelet activating factor (PAFR). Data obtained with Barbadin, an AP2 inhibitor able to impair endocytosis of many GPCRs, clearly show that internalization of ligand-bound FPR2 occurs independently of β-arrestin. In addition, a lipopeptide (pepducin) suggested to be a putative Gαq-inhibitor, is shown to lack inhibitory effect of the neutrophil response mediated by Gαq linked PAFR, but instead distinctly modulates the function of both FPR2 and the free fatty acid receptor FFAR2, two Gαi-coupled neutrophil GPCRs.

In conclusion, this thesis adds new knowledge and novel insight into FPR2 signaling in neutrophils and GPCR regulation mechanism in general. Hopefully this knowledge will contribute to future drug development for treating inflammatory diseases.

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