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Effect of intracellular loop 1 pepducins on ligand-receptor binding kinetics at the CXCR4 receptor

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Introduction. Pepducins are a class of lipidopeptide ligands derived from the intracellular loops of a GPCR and have been reported to act as allosteric modulators, although their exact mechanism remains unclear.[1] Pepducins for the chemokine receptor, CXCR4, have been described to act as positive or negative allosteric modulators and can exhibit agonist activity in the absence of the endogenous ligand CXCL12.[2] In this study, we investigated the interaction and mechanism of intracellular loop 1 pepducins with CXCR4.

Method. Experiments were performed in HEK293 cells stably expressing human CXCR4 tagged with the luciferase, NanoLuc, on its N-terminus (NL-CXCR4) or C-terminus (CXCR4-NL). The binding and displacement of fluorescently labelled CXCR4 ligands was studied in a NanoBRET assay[3] at equilibrium or as kinetic experiments reading once every minute using NL-CXCR4 or CXCR4-NL cells or membranes quantified on a PHERAstar FS plate reader (BMG).

Results. The affinity of fluorescent CXCL12 (CXCL12-red) was determined in NL-CXCR4 cells and membranes using NanoBRET saturation binding (pK_d(Cells)=7.15±0.04, n=5; pK_d(Membranes)=7.61±0.10, n=5) or alternatively the association binding kinetics in cells using 1.5625 to 100nM (pK_d=7.10±0.18, n=7) were analysed. Competition binding experiments in membranes showed that CXCL12-red binding was inhibited by pre-incubation with small molecules AMD3100 (pKi=7.03±0.04, n=5), IT1t (pKi=8.07±0.02, n=5) and CXCR4 pepducin ATI-2341 (pKi<6, maximal inhibition 58.3±2.2% (10µM), n=5). Control pepducins missing a lipid tail or the last three amino acids did not displace CXCL12-red at concentrations up to 10 µM. Displacement of CXCL12-red by ATI-2341 was followed in a kinetic assay in cells. ATI-2341 (10µM) shows displacement of CXCL12-red which reaches a plateau of 53.47±0.98% of the initial signal within 10 minutes (k_{off}=0.43±0.04 min^{-1}, n=5). ATI-2341f, a TAMRA-labelled ATI-2341, was used in the membrane binding assay to detect interaction with the Nluc-CXCR4 receptor. ATI-2431f showed no displaceable binding towards the N-terminal labelled NL-CXCR4, but did interact with the C-terminal CXCR4-NL. In CXCR4-NL cells, binding of ATI-2341f reached saturation after 15 min and displacement with high concentrations of ATI-2341 was possible within 10 minutes. AMD3100, IT1t and CXCL12 are not able to displace ATI-2341f binding at concentrations up to 100 or 1 µM, respectively.

Conclusion. These data suggest that ATI-2341 interacts with CXCR4 from an allosteric site on the inner leaflet of the cell membrane. Moreover, ATI-2341 decreases the binding of CXCL12 to CXCR4 suggesting an influence on the orthosteric binding site.

Refrences

1.Covic et al.(2002). PNAS 99:643-648.
2.Tchernychev et al.(2010). PNAS 107:22255-22259.
3.Stoddart et al.(2015). Nature Methods 12:661 -663.